

Repellency of Essential Oils to *Frankliniella occidentalis* (Thysanoptera: Thripidae) as Affected by Type of Oil and Polymer Release

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ABSTRACT Eight essential oils [0.125–1.0% (vol:vol) in acetone] were separately deposited on leaf disks to evaluate their potential to repel western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), adult females. Two of the best-performing essential oils were incorporated into polymer matrices of methyl cellulose or alginate [0.5 or 1% (wt:vol)] to verify the potential of the polymer to extend repellency of oils over time (24–120 h). Results showed that at a concentration of 0.5%, *Thymus vulgaris* L. (common thyme) and *Satureja montana* L. (winter savory) were the most repellent essential oils. For these two treatments, no western flower thrips were counted on treated leaf disks 60 min after the start of the test. *T. serpyllum* and *O. compactum* also showed repellency values $\geq 90\%$ at this concentration. With both the alginate and methyl cellulose polymers, the incorporation of polymers into treatment solutions containing 0.5% concentrations of *S. montana* and *T. serpyllum* resulted in higher repellency compared with treatment solutions lacking these polymers for a minimum of 3 d. For the alginate polymer, differences associated with polymer concentrations were most dramatic. High repellency was maintained for 4 d when a 0.5% concentration of the alginate was used in combination with a 0.5% concentration of *S. montana*. The use of repellent oils with polymers that extend their repellency may prove useful for both pre- and postharvest applications in flower crops.

KEY WORDS essential oil, polymer, coating, thrip, *Frankliniella occidentalis*

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most serious insect pests of ornamental crops worldwide (Robb et al. 1988, Robb and Parrella 1991). Direct feeding damage causes loss of photosynthetic capacity, growth retardation, destruction of buds and flowers, and malformations of fruit (Rosenheim et al. 1990, Welter et al. 1990, Sedy and Koschier 2003). Western flower thrips is difficult to control because it is polyphagous, has cryptic behavior, and is resistant to many insecticides (Espinosa et al. 2002, Jensen 2000, Espinosa et al. 2002, Kirk and Terry 2003, Bielza 2008).

Originating in North America, western flower thrips is the most common thrips species in California and Arizona (Bryan and Smith 1956, Mound and Kibby 1998). Until 1960, the distribution of this insect was restricted to western North America and Mexico. It was found in central and eastern North America in the early 1980s. At about the same time, it became established in Europe (Mantel and van de Vrie 1988). This

species was first recorded in Quebec, Canada, in 1986 (Kirk and Terry 2003). In Hawaii, the first infestation was reported on the island of Kauai in 1955, and the species has since spread to all other islands (Waterhouse and Norris 1989). Currently, western flower thrips is a major worldwide pest of agricultural and horticultural crops (Kirk and Terry 2003).

Western flower thrips has been found to be the most common species infesting orchid blossoms in certain areas of Hawaii, and western flower thrips is also an important pest of orchids in other areas of the world (Hata et al. 1993, Hara and Hata 1999, Hollingsworth et al. 2002). Thailand, Singapore, Taiwan, and Hawaii are the world's largest producers of orchids, especially orchids in the *Dendrobium* genus (Uchida 1994). Cut and potted orchids in Hawaii were valued at US\$18.2 million in 2008 (NASS 2011). These orchids are sold locally to other nurseries or hobbyists and are also exported nationally and internationally. However, the majority of cut *Dendrobium* spp. are shipped to the continental United States (Uchida 1994, Hollingsworth et al. 2002). Shipped flowers must be free of all quarantine pests. If pests are found in the exported plants at the point of destination, the quarantine inspectors will reject and send back or destroy the flower shipments (Hollingsworth et al. 2002). Results

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of a survey showed that growers of cut orchids in Hawaii considered thrips their most serious insect pest and frequently targeted these insects with pesticide sprays (Hollingsworth et al. 2000). Because thrips populations are difficult to control and individual thrips are difficult to see, there is a risk that infested flowers will be accidentally shipped to overseas destinations. If a chemical were available which could be sprayed on the harvested commodity to repel thrips immediately before shipment, this would reduce the risk that quarantine pests would be accidentally included with shipments of orchids exported overseas. The ideal chemical would not harm the delicate flowers, have a pleasant smell, and be safe to applicators.

Aromatic plants have been cultivated since antiquity for their organoleptic properties and have been used as spices, pot herbs, and medicinal herbs (Regnault-Roger 1997). These compounds confer a characteristic flavor and odor and are concentrated by steam distillation of plant foliage and other plant parts, resulting in volatile fragrant compounds commonly called "essential oils" (Regnault-Roger 1997, Isman 2000, Ibrahim et al. 2001). Essential oils generally consist of several constituents produced as secondary metabolites, the majority of which are hydrocarbons, terpenes, and polyphenolic compounds. The use of essential oils for pest management is relatively recent, and many new applications are under investigation (Regnault-Roger 1997, Isman 2000, Ibrahim et al. 2001, Nerio et al. 2010). Because of the high volatility of essential oils, direct application onto leaves has often been found to have limited benefits. An improved application method is to incorporate the oils into polymer mixture coatings to protect the bioactivity of the active compounds, obtain a better distribution, and maintain high concentrations of active compounds on the surface of the leaves for longer period.

Here, we used petri dish choice bioassays to explore the potential of using essential oils to repel western flower thrips adults. Our first objective was to determine which essential oils from our selected list were most repellent when applied to treated leaf disks (experiment 1). Our second objective was to test whether the period of repellency could be extended if the essential oils applied to leaf disks were incorporated into polymer matrices of methyl cellulose or alginate (experiment 2).

Materials and Methods

Insects, Plants, and Bioassay Arenas. Western flower thrips adults used in bioassays were from a laboratory colony started in 2008 at the U.S. Pacific Basin Agricultural Research Center in Hilo, HI. They were reared on pods of green beans (*Phaseolus vulgaris* L.) purchased from local supermarkets and held in ventilated plastic containers in the laboratory ($\approx 23 \pm 2^\circ\text{C}$). Streaks of honey were used as a food supplement, and beans containing thrips eggs were removed to fresh containers to obtain thrips adults of known age. Adult females < 2 wk in age were selected for bioassays. Petri dish arenas (90 by 22 mm) were ven-

tilated by cutting three 10-mm holes into the dish lid and covering these with silk screen inserts glued into place using contact cement (DAP Inc., Baltimore, MD) (Fig. 1). A 1.5% Bacto Agar (BD Biosciences, Franklin Lakes, NJ) mixture was poured into the bottom of the dish. Leaf disks (20 mm in diameter) cut from fully expanded leaves of seedling string bean plants ('Kentucky Blue') were positioned in the agar while it was still warm. The agar held the disks in place, provided moisture for leaf disks, and eliminated any spaces underneath in which thrips could hide.

Essential Oils, Polymer Syntheses, and Preparation of Treatment Solutions. Essential oils tested in repellency bioassays were extracted from lemon [*Citrus limon* (L.) Burm.f.], wintergreen (*Gaultheria procumbens* L.), peppermint (*Mentha* \times *piperita* L.), oregano (*Origanum compactum* Benth.), winter savory (*Satureja montana* L.), wild thyme (*Thymus serpyllum* L.), and common thyme (*Thymus vulgaris* L.). All essential oils were obtained from Pranarom International (Ghislenghien, Belgium). In addition to essential oils, we tested *d*-limonene (technical grade, FL Chemical Company, Inc., Winter Haven, FL). For synthesis of the alginate polymer, sodium alginate (from brown algae; molecular weight, 1.5×10^5 Da; low viscosity; 67% L-guluronic acid), ninhydrin reagent, and D-glucosamine were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Polymer modification (*O*-acylation of alginate) was carried out according to a method developed in our laboratory. An amount of 5 g of alginate was dissolved in 600 ml of distilled water at room temperature. The mixture was stirred overnight to ensure a complete solubility. Thereafter, the pH of solutions was adjusted to 7.5 by slow addition of 0.1 M NaOH, and the volume was adjusted to ≈ 800 ml. The acylation reactions were carried out by adding palmitoyl chloride (density, 0.907 g/ml) to polymer solutions in a ratio of 1:4 (wt:wt), maintaining the pH at 7.5 with NaOH 0.5 M, at 80°C . After 1 h, the reaction media were neutralized (pH 6.8–7.0) and precipitated with acetone. The precipitate, collected by filtration, was washed at 50°C with an excess of methanol and decanted. The washing was repeated twice to eliminate free fatty acids. Finally, the resulting functionalized product (*O*-palmitoylated alginate) was dried with pure acetone and in a ventilated oven at 40 – 50°C for 24 h (Han et al. 2008). For preparing the 1% alginate treatment solutions that did not contain essential oils, the formulation described above (consisting of 2% modified sodium alginate and 98% nonmodified sodium alginate) was mixed with distilled water only. Alginate–oil mixtures were made by first mixing together essential oil and Tween 80 (polysorbate 80, Thermo Fisher Scientific, Waltham, MA) emulsifier in a 1:2 ratio and then adding the premixed aqueous alginate solution to achieve a 0.5 or 1% alginate mixture having 0.5% essential oil. The 1% aqueous methyl cellulose suspension was prepared by first dissolving the methyl cellulose powder in water while heating up to 60°C for pregelatinization, followed by cooling in an ice bath until transparent, adding 0.25% glycerol as a plasticizer agent, and then adding 0.5% canola oil as a

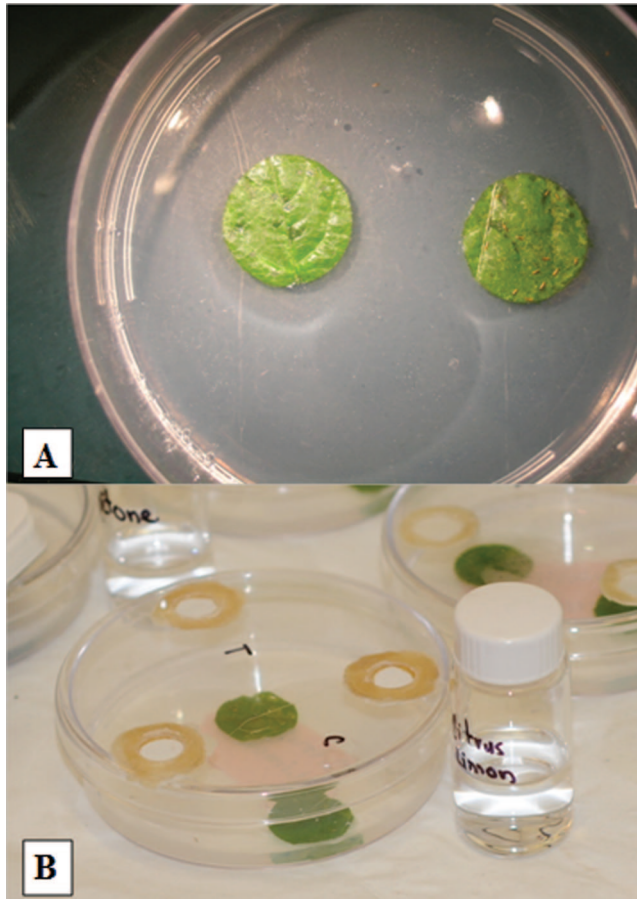


Fig. 1. Leaf disk bioassay arena. (A) Lid has been removed; note adult western flower thrips on leaf disk on right. (B) Three ventilation holes in the lid. (Online figure in color.)

hydrophobic agent. Then, the methyl cellulose mixture was either left as is (as a 1% methyl cellulose mixture) or further diluted with water (if a 0.5% methyl cellulose solution was desired) before mixing in the essential oil and Tween 80 (latter two premixed in a 1:2 ratio) to achieve a 0.5 or 1% methyl cellulose mixture having 0.5% essential oil. Finally, these mixtures containing methyl cellulose were mixed for 1 min using an Ultra-Turrax T25 dispersing machine (IKA Works, Wilmington, NC) at 25,000 rpm for 1 min, at room temperature.

Bioassay Setup. Experiment 1 tested repellency of essential oils without polymers. A pipette was used to apply 100 μ l of a solution containing an essential oil dissolved in acetone to each treated leaf disk. The tip of the pipette was used to spread the liquid evenly over the leaf disk surface. Each dish also held a leaf disk treated with acetone alone. For each essential oil or terpene, concentrations of oil in acetone tested were 0.125, 0.25, 0.5, and 1.0% (vol:vol). After solutions had dried, 20 western flower thrips adults that had been aspirated were shaken into the center area of the dish holding the agar and the two leaf disks. Five to seven replications of each treatment were carried out over

time. Dishes were held \approx 23°C in a fume hood illuminated with fluorescent lights. Experiment 2 tested the ability of alginate of methyl cellulose polymers to extend the period of repellency associated with two of the best performing oils (*S. montana* and *T. serpyllum*) identified in experiment 1. Procedures for infesting and holding petri dishes were the same as used in experiment 1. However, each dish held four leaf disks arranged in the shape of a “+” symbol, including two identically treated and two untreated disks. Treated leaf disks were positioned opposite from one another in the dish. Three types of treatment solutions were used: 1) an aqueous solution containing 1% methyl cellulose or 1% alginate alone (no essential oils); 2) an aqueous solution containing one of these polymers at a 0.5 or 1% concentration in combination with a 0.5% concentration of *S. montana* or *T. serpyllum*; and 3) an acetone solution containing a 0.25% solution of *S. montana* or *T. serpyllum*. For aqueous solutions, 100 μ l was applied to the leaf disk. Treatment solutions using acetone as the solvent were applied at 200 μ l per leaf disk. This volume (concentration) facilitated adequate spreading of the liquid across the leaf disk surface while keeping surface deposition rates of oils

Table 1. Proportion (SE, number of dishes, and total thrips) of all thrips on leaf disks found on the control leaf disk after 60 min

| Oil (Tukey's HSD) | Concn | | | |
|----------------------------------|----------------------------------|-----------------------|-----------------------|-----------------------|
| | 0.125% | 0.25% | 0.5% | 1.0% |
| <i>C. limon</i> (D) ^a | 0.60a (0.18, 3, 17) ^b | 0.32b (0.11, 7, 47) | 0.62b (0.05, 5, 39) | 0.48c (0.12, 3, 23) |
| <i>G. procumbens</i> (ABC) | 0.53a (0.05, 4, 23) | 0.65ab* (0.04, 4, 24) | 0.78ab (0.11, 5, 40) | 0.94ab* (0.06, 4, 26) |
| Limonene (CD) | 0.54a (0.10, 4, 33) | 0.50ab (—, 1, 8) | 0.53b (0.09, 4, 27) | 0.66bc (0.10, 6, 42) |
| <i>M. × piperita</i> (BCD) | 0.55a (0.10, 3, 20) | 0.64ab (0.08, 4, 30) | 0.77ab* (0.06, 5, 37) | 0.78ab* (0.06, 6, 49) |
| <i>O. compactum</i> (AB) | 0.64a (0.11, 4, 22) | 0.75ab* (0.0, 3, 20) | 0.93a* (0.04, 4, 32) | 1.0a* (0.0, 4, 25) |
| <i>S. montana</i> (A) | 0.51a (0.09, 2, 12) | 0.83a* (0.07, 5, 35) | 1.0a* (0.0, 3, 13) | 0.97ab* (0.03, 3, 19) |
| <i>T. serpyllum</i> (AB) | 0.75a (0.09, 4, 21) | 0.76ab (0.16, 3, 28) | 0.90a* (0.04, 7, 48) | 0.75ab (0.10, 4, 26) |
| <i>T. vulgaris</i> (AB) | 0.55a (0.10, 4, 25) | 0.61ab (0.09, 6, 41) | 1.0a* (0.0, 3, 25) | 1.0a* (0.0, 3, 29) |

Each 2.0-cm leaf disk was treated with 100 μ l of a solution with acetone as the solvent. Twenty adult female thrips were added to each replicate (Petri dish). Data were not used if fewer than four thrips were counted on both leaf disks in the dish. Within each column, means followed the same letter are not significantly different ($P = 0.05$; Tukey's HSD multiple comparison test). An asterisk (*) after the mean indicates that the 95% CL for the mean proportion did not overlap 0.50, the expected value in the absence of repellency or attraction.

^a Tukey's HSD means separation test designation. Oils followed by different letters (having none in common) are significantly different at $P \leq 0.05$ based on least significant means differences across all concentration levels.

^b Sum of thrips counted on all leaf disks within Petri dishes.

equivalent to those using aqueous formulations. For each polymer, two separate experiments were carried out during different weeks, by using three replicates of each treatment per experiment (testing six replicates per treatment in total). Because alginate and methyl cellulose bioassays were carried out at different times, data associated with these two polymers were analyzed separately.

Data Collection and Analysis. To determine repellency, thrips were counted on treated and untreated leaf disks without opening dishes. This was done after 60 min in experiment 1 (test to determine most effective oil), and after 24, 48, 72, 96, and 120 h in experiment 2 (test to determine whether polymers extend period of repellent activity to oil). The dependent variable used in analyses was number of thrips on experimental control disk(s) divided by sum of thrips on both experimental control disk(s) and treatment disk(s). This proportion, defined for convenience as "repellency," was arcsine square-root transformed (Steel and Torrie 1980) before being analyzed by analysis of variance (ANOVA) (test 1) and ANOVA and multivariate analysis of variance (MANOVA) (test 2) (SAS Institute 2008, 2010). Data were not used if fewer than four thrips were present on both treated and experimental control disk(s) or if mold was present on one or more leaf disks (a situation which occasionally occurred in experiment 2). The independent variables used in the ANOVA model for experiment 1 data were oil type, concentration (as a continuous variable), and an interaction term. Tukey's honestly significant difference (HSD) test was carried out on least squares means for the main effect of each oil over all concentrations tested. In addition, separate one-way ANOVA and Tukey's HSD analyses were carried out within each concentration level by using oil type as the independent variable. These separate analyses, although inflating the chance of type I errors over all comparisons, were justified by the finding of a significant interaction between oil type and concentration level. The 95% confidence intervals for Tukey means in these separate tests were used to determine whether the mean was significantly different from 0.50

(the expected proportion in the absence of repellency or attraction).

For experiment 2, data involving polymers were analyzed separately for alginate and methyl cellulose polymers using a MANOVA repeated measures approach (SAS Institute 2010). Dependent variables were the arcsine square root transformed proportion of thrips on leaf disks that were counted on control leaf disks at intervals of 24, 48, 72, 96, and 120 h after the time the dish was first set up. Differences among responses for different times of measurements were fit with an orthogonal polynomial. Independent variables were polymer concentration (0, 0.5, or 1.0%) (treated as an ordinal variable), oil type (*S. montana* or *T. serpyllum*), and the interaction of these two variables. To preserve orthogonality and simplify interpretation of results, treatments using 1% alginate alone or 1% methyl cellulose alone were omitted from analyses. In addition to this MANOVA test, treatments within each time period (24, 48, 72, 96, or 120 h) were analyzed separately using ANOVA followed by Tukey's HSD multiple comparison tests (SAS Institute 2008). The 95% confidence intervals for Tukey means were used to determine whether the mean was significantly different from 0.50 (the expected proportion in the absence of repellency or attraction).

Results

Experiment 1. The repellency of most of the essential oils progressively increased as the concentration of essential oils applied to treatment leaf disks increased from 0.125 to 0.5% (Table 1). ANOVA revealed that the type of oil, the concentration of oil and their interaction were all significant terms in the ANOVA model ($P \leq 0.01$ for all three variables) (Table 2). Over all concentrations, the most repellent oils were *G. procumbens*, *O. compactum*, *S. montana*, *T. serpyllum*, and *T. vulgaris*. *C. limon* and limonene were the least repellent, and *M. piperita* was intermediate (Tukey's HSD test for least squares means, Table 1). The oils that performed best at 0.5% (the lowest concentration that produced 100% repellency for the most

Table 2. Analysis of variance table for the proportion of all thrips on leaf disks found on the control leaf disk after 60 min. Data were arcsine square-root transformed before analysis

| Source | df | Mean square | F ratio | Prob. > F |
|-------------|-----|-------------|---------|-----------|
| Oil | 7 | 0.618 | 9.31 | <0.0001* |
| Concn | 1 | 2.609 | 39.33 | <0.0001* |
| Concn × oil | 7 | 0.182 | 2.75 | 0.01* |
| Error | 114 | 0.066 | | |

Asterisk (*) indicates significance.

effective oils) were *S. montana* and *T. vulgarens*, each having no western flower thrips counted on treated leaves 60 min after the start of the test. *T. serpyllum* and *O. compactum* also performed well and were both associated with repellency values ≥0.90 at this concentration. On the low end, the repellency of *C. limon* and limonene never exceeded 0.66, even at concentrations of 1% (Table 1).

Experiment 2. When 0.5% concentrations of *S. montana* and *T. serpyllum* oils were applied to leaf disks within 1% polymer matrices made of alginate, close to 100% repellency was observed over the first 48-h period. This was also true for *S. montana* applied in a 0.5% concentration of alginate (Table 3). Repellency of these three best performing treatments remained high for the first 3 d, and in the case of *Satureja montana* in a 0.5% concentration of alginate, the repellency never went below 97% over the first 4 d (Table 3). With only one exception, repellency values for treatments using *S. montana* and *T. serpyllum* without alginate polymer were always lower than treatments by using these same oils with the alginate, as measured on each of the first 4 d of the 5-d test. The exception was that the *T. serpyllum* no alginate treatment had higher repellency (91%) than the treatment using *T. serpyllum* with 0.5% alginate (repellency, 80%) as measured 24 h posttreatment (Table 3). Between 96 and 120 h, repellency decreased in all but one treatment (*S. montana* in acetone alone), indicating that treatment effects had effectively run their course over time. Interestingly,

Table 3. Proportion (SE, number of dishes, and total thrips) of all thrips on leaf disks counted on the control leaf disks 24–120 h after treatment with solutions containing oils alone, alginate alone, or oils emulsified within a solution containing alginate

| Treatment | Hours posttreatment | | | | |
|-------------------------------|-----------------------------------|-----------------------|-----------------------|------------------------|----------------------|
| | 24 | 48 | 72 | 96 | 120 |
| Alg 1% + SM ^a 0.5% | 0.97a* (0.02, 6, 91) ^b | 0.97a* (0.02, 6, 93) | 0.88a* (0.07, 4, 51) | 0.63bcd (0.13, 4, 35) | 0.53ab (0.07, 4, 28) |
| Alg 1% + TS ^c 0.5% | 1.0a* (0.00, 6, 111) | 0.99a* (0.01, 6, 100) | 0.88a* (0.05, 6, 73) | 0.85ab* (0.05, 6, 60) | 0.54ab (0.15, 4, 32) |
| Alg 0.5% + SM 0.5% | 0.98a* (0.02, 6, 115) | 1.0a* (0.0, 6, 101) | 0.97a* (0.03, 3, 43) | 1.0a* (0.0, 3, 29) | 0.77ab (0.10, 2, 17) |
| Alg 0.5% + TS 0.5% | 0.80ab (0.12, 4, 51) | 1.0a* (0.0, 5, 83) | 0.97a* (0.03, 5, 64) | 0.78abc* (0.05, 5, 52) | 0.74a* (0.08, 5, 31) |
| Alg 1% | 0.86ab* (0.06, 6, 114) | 0.83b* (0.03, 6, 108) | 0.81ab* (0.06, 6, 87) | 0.51cd (0.05, 6, 75) | 0.35b* (0.04, 6, 47) |
| SM 0.25% | 0.65b (0.12, 5, 58) | 0.60c (0.08, 5, 75) | 0.56b (0.08, 5, 74) | 0.49cd (0.12, 4, 64) | 0.52ab (0.06, 5, 42) |
| TS 0.25% | 0.91ab* (0.03, 6, 86) | 0.67bc (0.07, 6, 87) | 0.57b (0.07, 6, 82) | 0.40d* (0.04, 6, 81) | 0.25c* (0.07, 6, 57) |

Each 20-mm leaf disk was treated either with 100 μl of aqueous solution (used when the mixture contained alginate) or 200 μl of a solution with acetone as the solvent if alginate was not present (using one-half the concentration of oil to keep surface deposition rates constant). Twenty adult female thrips were added to each replicate (Petri dish). Data were not used if fewer than four thrips were counted on all four leaf disks in the dish or if mold was present on leaf disks. Within each column, means followed the same letter are not significantly different (*P* = 0.05; Tukey's HSD multiple comparison test). An asterisk (*) after the mean indicates that the 95% CL for the mean proportion did not overlap 0.50.

^a Oil of *S. montana*.

^b Sum of thrips counted on all leaf disks within Petri dishes.

^c Oil of *T. serpyllum*.

Table 4. MANOVA table for the proportion of all thrips on leaf disks found on the control leaf disks 24–120 h after treatment with oils alone, or oils emulsified within a solution containing alginate

| Source | df | Test | Value | Prob. > F |
|----------------------------|----|----------------|-------|-----------|
| Model | 24 | | | |
| Intercept | 4 | F | 1.36 | <0.008* |
| Polymer concn ^a | 8 | Pillai's trace | 0.74 | <0.04* |
| Oil type | 4 | F | 3.94 | 0.02* |
| Polymer concn × oil type | 8 | Pillai's trace | 0.70 | 0.06* |
| Error | 18 | | | |

Data were arcsine square-root transformed before analysis. Differences among responses for different days of measurement were fit with an orthogonal polynomial.

^a Modeled as an ordinal value of 0, 0.5, or 1.0%. To prevent bias of results across polymer concentration levels, the alginate alone treatment was omitted from the model.

over the first 3 d, the 1% alginate alone treatment was also more repellent than either of the essential oil treatment used alone and the repellency value for the 1% alginate alone treatment for each of these 3 d was significantly >0.50, the expected proportion in the absence of repellency or attraction (Table 3). On day 5 (120 h after treatment), repellency values in treatments using 1% alginate alone or *T. serpyllum* in acetone alone were 0.35 and 0.25, respectively. This was significantly below the proportion of 0.50, indicating no feeding preference between the treated and experiment control leaf disks (Table 3).

The MANOVA for the data involving the alginate polymer showed that polymer concentration and oil type were significant terms in the statistical model, with *P* values of 0.04 and 0.02, respectively. The interaction of these two variables was marginally significant at *P* = 0.06 (Table 4). Graphically, the main effects of incorporating 0.5 or 1.0% alginate in treatment solutions containing *S. montana* and *T. serpyllum* is depicted in Fig. 2A. For data collected at 48, 72, and 96 h, repellency values were ≥30% higher if these oils were used in combination with the alginate polymer at either concentration (Fig. 2A). From 72–120 h,

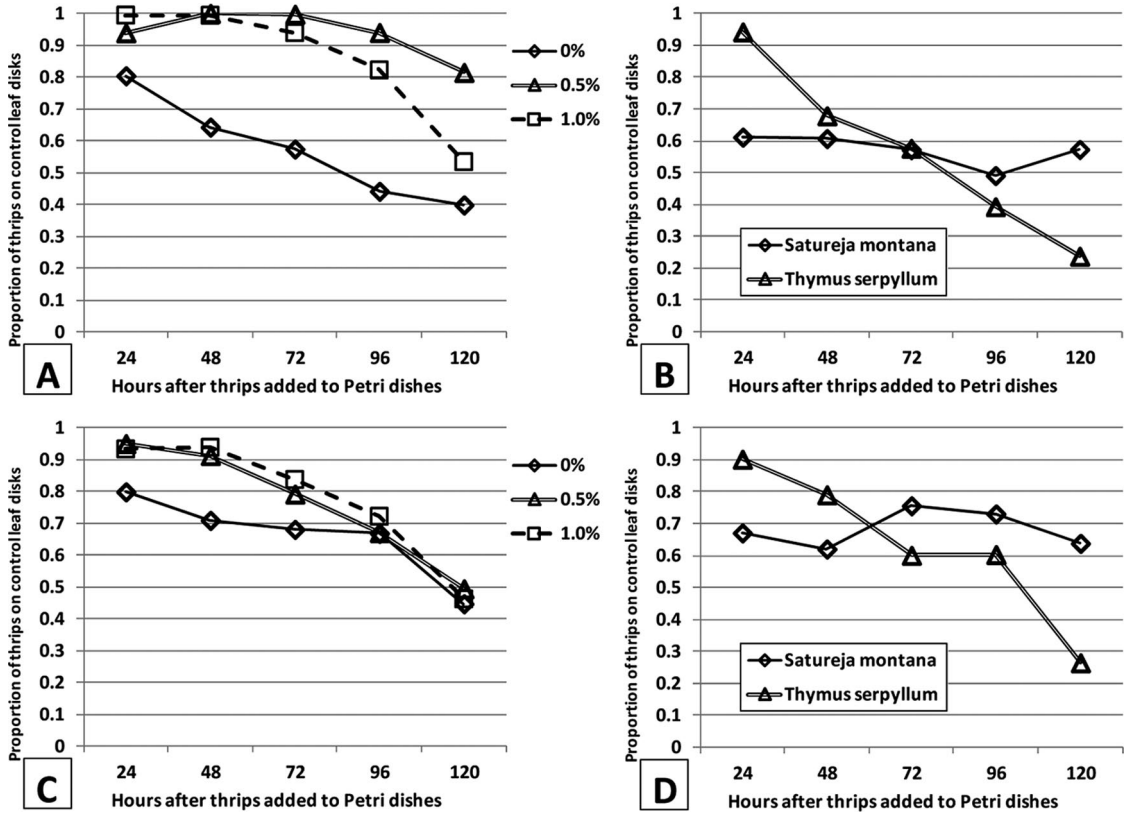


Fig. 2. Least squares means (back-transformed) showing time trends for main effect of alginate polymer concentration (0, 0.5, or 1.0%) on repellency values across 0 and 0.5% concentrations of *S. montana* and *T. serpyllum* (A); main effect of each of these two oils across all three alginate polymer concentrations (B); main effect of methyl cellulose polymer concentration (0, 0.5, or 1.0%) on repellency values across concentrations of 0 and 0.5% concentrations of *S. montana* and *T. serpyllum* (C); and main effect of each of these oils across all three methyl cellulose polymer concentrations (D).

repellency progressively decreased across all concentrations of alginate, although it did not go below an average of 80% in the case of alginate used at a concentration of 0.5% (Fig. 2A). Averaging across all alginate polymer concentration levels (0, 0.5, and 1.0%), *T. serpyllum* was more repellent than *S. montana* at 24 h (94 versus 61%, respectively); was similar to *S. montana* at 48, 72, and 96 h; and was less repellent at 120 h (24 versus 57%, respectively) (Fig. 2B). However, given that the interaction between polymer concentration and oil type was marginally significant, it is important to examine results for each treatment individually to understand the dynamics producing this main effect.

Table 5 shows results for the methyl cellulose polymer. Overall, repellency values of oils were slightly lower in association with this polymer compared with the alginate and within-treatment variability was greater, resulting in fewer statistically significant treatment differences. Nevertheless, high repellency ($\geq 94\%$) was associated with *T. serpyllum* over the first 48 h when these were present within a methyl cellulose matrix of with 0.5 or 1%. In the absence of methyl cellulose, *T. serpyllum* was less repellent (repellency values of 0.70 and 0.79 for 24 and 48 h, respectively),

and this difference was significant in the case of 24 h. Over the first 4 d of the experiment, the treatment using methyl cellulose alone was associated with repellency values between 0.76 and 0.80, and in all cases, these values were significantly >0.50 (the expected value in the absence of repellency/attraction) (Table 3).

The MANOVA for the data involving the methyl cellulose polymer (Table 6) showed that neither polymer concentration, oil type nor their interaction was a significant term in the statistical model (all P values >0.05). Graphically, the main effects of incorporating 0.5 or 1.0% methyl cellulose in treatment solutions containing *S. montana* and *T. serpyllum* is depicted in Fig. 2C. The pattern observed was similar to the pattern observed for the alginate polymer (i.e., repellency was lowest for the 0% concentration level of the polymer and similar for the 0.5 and 1.0% polymer concentration levels) (Fig. 2C). A comparison of the repellency of *S. montana* versus *T. serpyllum* averaged over polymer concentration levels is depicted in Fig. 2D. The pattern observed was very similar to the pattern observed in the alginate data: *T. serpyllum* was initially more repellent (90% repellency at 24 h), but lost repellency more rapidly over time (26% repel-

Table 5. Proportion (SE, number of dishes, and total thrips) of all thrips on leaf disks found on the control leaf disks 24–120 h after treatment with oils alone, methyl cellulose (MC) alone, or oils emulsified within a solution containing methyl cellulose

| Treatment | Hours posttreatment | | | | |
|------------------------------|-------------------------------------|-----------------------|----------------------|----------------------|---------------------|
| | 24 | 48 | 72 | 96 | 120 |
| MC 1% + SM ^a 0.5% | 0.93ab* (0.93, 6, 107) ^b | 0.96a* (0.02, 5, 71) | 0.86a* (0.07, 5, 68) | 0.74a* (0.01, 3, 31) | 0.39a (0.06, 3, 29) |
| MC 1% + TS ^c 0.5% | 0.97a* (0.3, 6, 104) | 0.95a* (0.03, 5, 86) | 0.89a* (0.06, 5, 77) | 0.69a (0.10, 3, 27) | 0.53a (0.21, 3, 26) |
| MC 0.5% + SM 0.5% | 0.91ab* (0.05, 6, 104) | 0.87a* (0.06, 6, 80) | 0.75a (0.16, 5, 65) | 0.59a (0.26, 3, 27) | 0.45a (0.23, 3, 19) |
| MC 0.5% + TS 0.5% | 0.96ab* (0.02, 6, 99) | 0.94a* (0.02, 6, 89) | 0.81a* (0.09, 6, 74) | 0.69a (0.14, 6, 62) | 0.52a (0.13, 5, 48) |
| MC 1% | 0.77abc* (0.10, 6, 80) | 0.80ab* (0.01, 6, 79) | 0.76a* (0.08, 5, 66) | 0.76a* (0.07, 5, 51) | 0.65a (0.09, 5, 56) |
| SM 0.25% | 0.60c (0.10, 6, 82) | 0.56b (0.09, 6, 88) | 0.70a (0.08, 6, 81) | 0.72a (0.09, 4, 41) | 0.64a (0.05, 4, 45) |
| TS 0.25% | 0.70bc (0.10, 6, 75) | 0.79ab* (0.04, 3, 52) | 0.59a (0.16, 3, 51) | 0.60a* (0.01, 3, 53) | 0.27a (0.10, 3, 38) |

Each 20-mm leaf disk was treated either with 100 μl of aqueous solution (used when the mixture contained methyl cellulose) or 200 μl of a solution with acetone as the solvent if methyl cellulose was not present (using one half of the concentration of oil to keep surface deposition rates constant). Twenty adult female thrips were added to each replicate (Petri dish). Data were not used if fewer than four thrips were counted on all four leaf disks in the dish or if mold was present on leaf disks. Within each column, means followed the same letter are not significantly different ($P = 0.05$; Tukey's HSD multiple comparison test). An asterisk (*) after the mean indicates that the 95% CL for the mean proportion did not overlap 0.50.

^a Oil of *S. montana*.

^b Sum of thrips counted on all leaf disks within Petri dishes.

^c Oil of *T. serpyllum*.

lency after 120 h). By contrast, repellency associated with *S. montana* was more stable, dropping from 67 to 63% over the same period (Fig. 2D).

Discussion

Our results demonstrate that the oils from *S. montana*, *T. vulgaris*, *T. serpyllum*, and *O. compactum* were highly repellent to western flower thrips, whereas the oil from *C. limon* and the terpene limonene were not repellent. This information is an important first step toward developing repellent postharvest sprays for flowers. Different oils and their terpene components vary dramatically in price, and it is reasonable to choose an oil or terpene that is acceptable for a particular use considering repellency, phytotoxicity and cost. For the four most effective essential oils mentioned above, *O. compactum* was found to be ≈23–25% less expensive than the other three oils from a commercial online supplier selling in bulk. Limonene is ≈10–20 times less expensive than any of these oils (Hollingsworth 2005) but unfortunately was not effective as a repellent.

Table 6. MANOVA table for the proportion of all thrips on leaf disks found on the control leaf disks 24–120 h after treatment with oils alone, or oils emulsified within a solution containing methyl cellulose

| Source | df | Test | Value | Prob. > F |
|----------------------------|----|----------------|-------|-----------|
| Model | 24 | | | |
| Intercept | 4 | F | 1.36 | <0.06 |
| Polymer concn ^a | 8 | Pillai's trace | 0.74 | <0.55 |
| Oil type | 4 | F | 3.94 | 0.14 |
| Polymer concn × oil type | 8 | Pillai's trace | 0.70 | 0.55 |
| Error | 15 | | | |

Data were arcsine square-root transformed before analysis. Differences among responses for different days of measurement were fit with an orthogonal polynomial.

^a Modeled as ordinal value of 0, 0.5, or 1.0%. To prevent bias of results across polymer concentration levels, the treatment using methyl cellulose alone was omitted from the model.

With both the alginate and methyl cellulose polymers, the incorporation of the polymer into treatment solutions containing 0.5% concentrations of *S. montana* and *T. serpyllum* resulted in higher repellency values for a minimum of 3 d. For the alginate polymer, differences associated with polymer concentrations were most dramatic and were also statistically significant. High repellency was maintained for 4 d when a 0.5% concentration of the alginate was used, and best results occurred when the 0.5% concentration of the alginate was paired with *S. montana* as opposed to oil of *T. serpyllum*. The latter lost repellency over time more rapidly than the former in both the alginate and methyl cellulose tests. In the test involving the alginate polymer, for each day of observation starting with day 2 (48 h posttreatment), repellency values for all treatments containing polymer + essential oil were higher than repellency values for treatments containing essential oil alone over the 5-d observation period. A portion of this increased repellency might have been due to the repellency of the alginate itself. However, although the alginate alone treatment seemed to lose its repellency after 72 h, the repellency of treatments that used a combination of oil + polymer remained for an additional 24 or 48 h (depending on the treatment) suggesting that the polymer slowed the loss of oil volatiles and extended their repellency. As noted, in several instances involving the alginate test it seemed that by 120 h posttreatment, treated leaf disks had actually become more attractive than the untreated leaf disks within the same dish, as evidenced by repellency values far below 0.50, the expected no-preference value. One possible explanation is that feeding and oviposition by the thrips on the experimental control leaf disks led to gradual reduction in relative attractiveness. At that point, the higher food quality of the treated leaf disks could have overcome any lingering repellent effects associated with treatments. In support of this hypothesis, it was noted that experimental control leaf disks were noticeably lighter green in color by the end of the 5-d observation period due

to the removal of chlorophyll from individual cells by thrips when feeding. In a field situation in which the insect can choose to leave the area of the treated plant material, the period of repellency associated with treatments of polymer + essential oil might be longer than the 3–4 d observed in these petri dish bioassays.

In the test using methyl cellulose as the polymer, results indicated that the polymer coating by itself was repellent, just as was found for the alginate. However, coatings made up of a mixture of the methyl cellulose polymer and essential oil were generally less repellent than corresponding mixtures prepared using the alginate polymer, and none of these polymer–oil mixtures were associated with repellency values ≥ 0.75 after day 3.

Alginate is an anionic polysaccharide composed of mannuronic acid and guluronic acid residues extracted from seaweed. This nontoxic polymer is biodegradable, biocompatible and is easily modified through physical or chemical methods (Han et al. 2008). It is used frequently for encapsulation applications due to its ability to form gels in the presence of divalent cations such as calcium by inotropic gelation. However, gel erosion is a common problem with alginate (Murata et al. 1993). To reduce this phenomenon, acylation by using fatty acid derivatives was done to enhance the hydrophobicity of the polymer (Le Tien et al. 2003) and protect against moisture (Han et al. 2008). It also has been demonstrated that the acylation of alginate can ensure the controlled release of active compounds (Le Tien et al. 2003) and provide protection from the enzyme (catalase) and bacteriocin activity (nisin) (Le Tien et al. 2004, Millette et al. 2007). Acylation of alginate can keep micronutrients stable during storage over 6 mo at 100% RH (Han et al. 2008) and provide resistance to probiotic bacteria under gastric conditions (Le Tien et al. 2004). However, using this technology to maintain repellency associated with essential oils is a new application.

In a literature review by Koschier (2008), a list of volatile compounds attractive to adult Thysanoptera has been compiled. The list shows that western flower thrips is attracted to various natural compounds including the monoterpenes (+)-citronellol, 1,8-cineole, geraniol, linalool, linalool oxide pyran, and nerol, and the sesquiterpene (*E*)- β -farnesene. Other attractants include benzaldehyde and its derivative compounds (C_6 – C_1), *p*-anisaldehyde, *o*-anisaldehyde, phenylpropanoid compounds (C_6 – C_3), eugenol, and 3-phelpropion-aldehyde. In addition, amines and other N-containing compounds such as ethyl isonicotinate, ethyl nicotinate, methyl isonicotinate, and methyl 4-pyridyl ketone are attractive to western flower thrips.

Perhaps because it is a polyphagous insect herbivore, relatively few compounds are known to repel western flower thrips. Methyl salicylate (found in oil of wintergreen) and salicylaldehyde are among these compounds (Koschier et al. 2000, Chermenskaya et al. 2001, Koschier et al. 2007). Methyl salicylate is a phenylpropanoid compound derived from salicylic acid

and is the primary constituent in the oil of wintergreen (Koschier et al. 2007). In our study, the source of wintergreen oil tested was *G. procumbens*, a plant commonly known as eastern teaberry or American wintergreen. Although this oil was highly repellent at the highest concentration tested (1%), it was relatively less repellent than certain other oils at lower concentrations.

Reitz et al. (2008) carried out field trials in tomato (*Solanum lycopersicum* L.) testing whether any of three different essential oils would reduce the spread of tomato spotted wilt (family *Bunyaviridae*, genus *Tospovirus*, TSW), vectored by *Frankliniella* spp. Oils tested were geraniol, lemongrass (*Cymbopogon flexuosus* Spreng.) oil and tea tree [*Melaleuca alternifolia* (Maiden & Betche) Cheel] oil. The oils were applied at 250 ppm (162.5 ml/ha) twice per week either alone or in combination with kaolin. In the absence of kaolin, the incidence of TSW was only slightly lower in plots treated with essential oils compared with the experimental control. However, in the presence of the kaolin, the incidence of TSW by the end of the season was much lower than found in the control plots, with a maximum reduction of 51% being associated with the use of tea tree oil (Reitz et al. 2008). One possible explanation for their results is that the kaolin slowed the rate of evaporation of the essential oils, extending the period of repellency.

Essential oils or their constituents also have potential utility in “push-pull” strategies. This integrated pest management tool uses a combination of stimuli to manipulate the distribution and abundance of insect pests, natural enemies, or both (Cook et al. 2007). The aim of this strategy is to facilitate pest control by concentrating the pest in a specific area. First, the pest is repelled or deterred away from the main crop (push) by using repellent or deterrent agent. Simultaneously, the pest is attracted (pull) to another area such as lure traps or trap crops that are highly attractive and where natural enemies can be found. The concentrated population of the pest facilitates control (Cook et al. 2007). This strategy is considered to have most potential in high-value horticultural production in enclosed environments (Cook et al. 2007, Koschier 2008).

In a glasshouse experiment in England, Bennison et al. (2002) attempted a push-pull strategy against western flower thrips. They successfully used a chrysanthemum (*Chrysanthemum indicum* L.) cultivar that was very attractive to western flower thrips to “pull” western flower thrips away from plants of a less attractive cultivar interspersed on greenhouse benches after enhancing the attractive cultivar by baiting plants with the plant volatile (*E*)- β -farnesene, known to be an attractant for western flower thrips. The predatory mites *Stratiolaelaps (Hypoaspis) miles* Womersley and *Gaeolaelaps (Hypoaspis) aculeifer* (Canestrini) were released on the chrysanthemum plants to increase western flower thrips predation. In separate olfactory tests, they found that rosemary leaves and volatiles were repellent to western flower thrips, but these were also repellent to the thrips

predator *Orius laevigatus* (Fieber), precluding their use. Although their results showed the potential of using a push-pull strategy, they remarked on the need to identify a suitable semiochemicals which have activity against western flower thrips but not biological control agents. Although rosemary (*Rosmarinus officinalis* L.) leaves and volatiles were repellent in their study, rosemary oil is rich in cineole, and it has been observed previously that cineole could be an attractant to western flower thrips (Koschier 2008). Presumably, the repellency of the essential oil from a particular plant species is influenced not only by variations in chemical constituents that are known to vary geographically but also according to the specific volatile concentration perceived by the insect.

Oregano (*Origanum* spp.), thyme (*Thymus* spp.), and savory (*Satureja* spp.) essential oils are rich in carvacrol and thymol (Harvala et al. 1987, Daferena et al. 2000, Radonic and Milos 2003, Sedy and Koschier 2003). *T. vulgaris* is known to be a strong repellent of the cigarette beetle, *Lasioderma serricorne* (F.) (Hori 2003). Carvacrol was found to be a successful repellent against mosquitoes when applied to the skin (Park et al. 2005). Our study has demonstrated that these essential oils are also repellent to western flower thrips. Our results are also in agreement with those of Chang et al. (2006), who showed that formulations based on creams or polymer mixtures for controlled release increased the duration of repellency.

This research was motivated by the desire to develop repellent sprays for use on orchid flowers after harvest to completely repel thrips from flowers. Although not reported here, we have carried out some preliminary spray tests of candidate mixtures. We sprayed to the point of runoff freshly harvested *Dendrobium* orchid stems with mixtures containing 1% alginate or methyl cellulose that also contained either 0.5% *S. montana* or *T. serpyllum*. These orchids were infested with western flower thrips either before or after spraying with the polymer-essential oil mixture). Results in terms of repellency were promising and no immediately phytotoxicity was observed. However, treated flower stems began senescing several days after spraying, whereas senescence was delayed for a week or more for flowers sprayed with water only. In future trials, concentrations, application rates, or both will be reduced in an effort to circumvent this problem.

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