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Biopesticide effects on pupae and adult mortality of *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract Reliance on broad spectrum insecticides for pest control has led to significant environmental damage, human health problems and rapid evolution of insect resistance. These shortcomings have caused a renewed interest in exploring biologically based pest control methods. Among these, the use of plant essential oils, hydrolates and other natural products offer a promising alternative to broad spectrum pesticides. Here, we explored the effect of several bioproducts on pupal and adult mortality (through contact and ingestion) of the two most important pest species of fruit production in South America, South American fruit fly, *Anastrepha fraterculus* and Medfly, *Ceratitis capitata*. Topical application of different bioproducts produced different effects on pupal mortality of *A. fraterculus* and *C. capitata.* Essential oils of *Baccharis dracunculifolia* and *Pinus elliottii,* both containing high proportions of α and β-pinene and limonene (among other compounds), caused 100% mortality on *C. capitata* pupae and strongly suppressed adult eclosion of *A. fraterculus* in comparison with controls. Extracts of *Solanum granulosum* plus *Ricinus communis* also caused a moderate yet significant reduction in adult eclosion when compared with the control. All other tested products had no effect on adult emergence. Toxicity on adults through ingestion was greatest for extracts of *S. granulosum* plus *R. communis*, followed by Spinosad (Flipper®) and *B. dracunculifolia* oil (although in these two cases survival was above 40%), while other tested products had no effect on adult mortality. We discuss future research and the potential use of some of the tested products as a component for rational pest management strategies.

Key words essential oils, hydrolates, limonene, plant extracts, rational pest management, *α*-pinene.

INTRODUCTION

Use and abuse of synthetic chemical products for pest control, since the discovery the insecticidal properties of DDT, has resulted in a series of negative side effects on human health and the environment that require the adoption of rational approaches for pest management (Damalas & Eleftherohorinos 2011). Pest management strategies based on the use of biopesticides offer a long-term alternative to conventional pest control (Copping & Menn 2000). A potential alternative to synthetic broad-spectrum pesticide use relies on exploiting insecticidal and other properties of plant compounds to develop botanical pest management tools (Isman 2000). Among these, essential oils (and in some cases hydrolates resulting from the extraction process) in some plant families are effective for controlling pests and to rapidly degrade in the environment (Petrakis *et al.* 2015; Tripathi *et al.* 2009). Plant essential oils, their derivatives and extracts have been found to repel or attract several species of insects and in some cases produce toxicity or alter fundamental physiological processes (Regnault-Roger *et al.* 2012).

Essential oils and plant extracts can be toxic to eggs, larvae, pupae, and adults of several species of insects of economic importance. Toxicity can be produced by direct contact, ingestion or fumigation (Regnault-Roger *et al.* 2012). In addition to their toxicity, some essential oils can act as repellents, attractants, oviposition stimulants or deterrents and in other cases, can enhance mating success of exposed adults (Shelly *et al.* 2007; Nerio *et al.* 2010).

The family Tephritidae (true fruit flies) is composed of more than 4500 species, all of them phytophagous, many of which are pests of economic importance for fruit production (Norrbom *et al.* 1999). Several tephritid pest species have been reported to be responsive to plant-derived oils and other chemicals. Essential oils of several plants have been found to be toxic to Medfly, *Ceratitis capitata* by exposure through direct contact, ingestion or fumigation (Benelli *et al.* 2012; Papachristos *et al.* 2009; de Oliveira *et al.* 2014). Interestingly, essential oils of some plant species in the genus *Tagetes* (Asteraceae) have been found to be toxic while also being attractive to males and repellent to females of *C. capitata* (López *et al.* 2011). Toxicity

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of citrus peel oils on *C. capitata* seems to be related to limonene content and to a lesser degree to α and β-pinene content (Papachristos *et al*. 2009). Essential oils, such as *Lavender angustifolia* and *Hyptis suaveolens*, are also toxic when ingested by the olive fly, *Bactrocera oleae* (Canale *et al*. 2013), and some essential oils can act as repellants or oviposition deterrents against the Queensland fruit fly, *Bactrocera tryoni* (Hidayat *et al*. 2013). In the case of fruit flies in the genus *Anastrepha*, citrus peel oils have been found to be toxic to neonate larvae of the Caribbean fruit fly, *Anastrepha suspensa*, and this toxicity was mainly caused by linalool and limonene content (Greany *et al*. 1983). In the case of the South American fruit fly *Anastrepha fraterculus*, citrus extracts, limonene and citral are toxic to eggs and larvae (Ruiz *et al*. 2014), while exposure to volatiles of fruit of some plants, such as eugenol present in pepper, cinnamon and laurel, can enhance or diminish sexual performance of exposed males (Vera *et al*. 2013).

C. capitata and *A. fraterculus* cryptic species complex constitute the most economically important group of insects affecting fruit production in South America (Uchôa 2012). Current field control strategies largely rely on use of broad-spectrum pesticides in conjunction with food baits that primarily target adults. Few viable alternatives currently exist to target immature stages (eggs, larvae and pupae) (Ovruski *et al*. 2016) or to replace broad-spectrum synthetic chemicals with effective environmentally friendly biopesticides.

In an effort to develop rational pest management tools for tephritid pests, we determined survival of pupae and adults of *A. fraterculus* and *C. capitata* exposed to contact and/or ingestion of different products of biological origin including some plant essential oils and hydrolates. We also compare the effect of the plant-derived products and Spinosad ingestion by adult flies, using water as a control.

MATERIALS AND METHODS

Source of flies

All *Anastrepha fraterculus* and *Ceratitis.capitata* individuals used in bioassays were obtained from previously established laboratory colonies held at the LIEMEN-PROIMI laboratories, Tucumán, Argentina. Both tephritid colonies had been held under artificial conditions of 26 ± 1°C, 60 ± 5% RH, and a 12:12 (L:D) h photoperiod for more than 15 years (~150 generations) and reared according to methods detailed in Braga-Sobrinho *et al*. (2006, 2010) and Vera *et al*. (2007). Both tephritid colonies were refreshed with wild flies every year. To carry out the assays, 2-day-old pupae of both species were transferred to an environmentally controlled chamber (26°C, 60% RH) until they reached the necessary age for the assays. Subsequently, they were conditioned as described below to comprise the different treatments.

Biopesticides

Five commercial biopesticides *Bacillus thuringiensis* (Baprom® – Delta endotoxin), *B. thuringiensis* var. Kurstaki (Biospam®), *Beauveria* SP® (Laboratory San Pablo, Tucumán, Argentina). Spinosad 1 (0.024/100 (g/mL) Flipper®] and Spinosad 2 [48/100 (g/mL.) Tracer®) and five botanically derived products (hydrolate of *Baccharis dracunculifolia* DC, *B. dracunculifolia* oil, *Pinus elliottii* oil, hydrolate of *P. elliottii* and a mixture of extract of *Solanum granulosoleprosum* + *Ricinus communis*) were evaluated in the present study.

Essential oil and hydrolate extraction

Essential oils (EOs) and hydrolates were obtained using a 600 kg biomass capacity stainless steel distiller steam fed with a water boiler, with an average vapour pressure of 2.5 kPa. Leaves and stems (up to 5 mm in diameter) of each plant species were chopped and aerated for 15 h and subjected to the passage of vapour for 6 h. After 80 minutes, distilled products were collected in a glass vial. Essential oils were then separated using hypodermic syringes, while the remaining supernatant product of oil extraction was the hydrolate.

The *Solanum* + *Ricinus* extract was obtained through alcohol extraction by placing a mix of *Solanum granulosoleprosum* and *Ricinus communis* (1:1, *w*/w) chopped fresh leaves in a 500 mL amber glass vial, covered with quaternary *Eucalyptus* alcohol and kept in a cool dark storage facility for 4 months. Product/water ratio for all products employed are detailed in Table 1.

Chemical analyses

Chemical composition of *Baccharis dracunculifolia* and *Pinus elliottii* oils was characterised at the Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba, using gas chromatography–mass spectrometry (GC–MS) with a Perkin Elmer Clarus 600 chromatograph and a Perkin Elmer DB5 capillary column (60 m × 0.25 mm × 0.25 μm). Data were acquired using TURBOMASS 5.4.2. software. Helium was used as carrier gas (341.98 Kpa). Injector temperature was set at 250°C, the GC oven was programmed at an initial temperature of 60°C (5 min) and increasing at a rate of 5°C/min to a final temperature of 240°C (10 min.). The chromatogram was obtained on scan mode from $m/z = 50$ to $m/z = 350$ (scan time: 0.2 s, inter-scan time: 0.1 s). Samples were diluted with hexane, and 1 μL was automatically injected to the chromatograph. The same procedure was used for the extract of *Solanum granulosoleprosum* + *Ricinus communis* but the sample failed to produce any signal on the GC–MS.

Effectiveness on pupal stage

Twenty 6-day-old pupae of *Anastrepha fraterculus* and *Ceratitis capitata* were placed into two Petri dishes (9.5-cm diameter) over a tissue paper and sprayed with 2 mL of each biopesticide. Treatments were *Bacillus thuringiensis* (Baprom® – Delta endotoxin) – 4.2% (T1); *B. thuringiensis* var. Kurstaki (Biospam®) – 3% (T2); *Beauveria* SP® – 2% (T3); Spinosad 1 (Flipper®) – 1.5% (T4) and Spinosad 2 (Tracer®) – 1.5% (T5); *Baccharis dracunculifolia* hydrolate – 35% (T6); *B. dracunculifolia* hydrolate – 50% (T7); *B. dracunculifolia* hydrolate – 75%

Table 1 Product (treatment) and dose (volume and (ratio)) for topical application on *Anastrepha fraterculus* and *Ceratitis capitata* pupae during Assay 1 and for consumption by adult flies in Assay 2

	Treatments	Product (mL)	Water (mL)	*v*/v (Ratio) (mL)
Pupae	T1 – *Bacillus thuringiensis* Delta endotoxin (Baprom®)	22	500	22:500 (1:22)
	T2 – *B. thuringiensis* var. Kurstaki (Biospam®)	15	485	15:485 (1:2)
	T3 – *Beauveria bassiana*	10	490	10:490 (1:49)
	T4 – Spinosad 1 (Flipper®)	3	200	3:200 (1:67)
	T5 – Spinosad 2 (Tracer®)	11	725	11:725 (1:6)
	T6 – *Baccharis dracunculifolia* hydrolate 1	175	325	175:325 (1:1.9)
	T7 – *B. dracunculifolia* hydrolate 2	250	250	250:250 (1:1)
	T8 – *B. dracunculifolia* hydrolate 3	375	125	375:125 (3:1)
	T9 – *Pinus elliottii* hydrolate 1	3	25	3:25 (1:8)
	T10 – *Pinus elliottii* hydrolate 2	20	25	20:25 (1:1.25)
	T11 – *B. dracunculifolia* oil	2	23	2:23 (1:11.5)
	T12 – *Pinus elliottii* oil	2	23	2:23 (1:11.5)
	T13 – *Solanum granulosoleprosum* + *Ricinus* 1	3	25	3:25 (1:8)
	T14 – *S. granulosoleprosum* + *Ricinus* 2	20	0	20:0 (Pure)
	T15 – Control (water)	0	20	0:20 (Pure)
Adult consumption	T1 – Spinosad 1 (Flipper)	3	200	3:200 (1:67)
	T2 – *B. dracunculifolia* hydrolate 1	175	325	175:325 (1:1.9)
	T3 – *Pinus elliottii* hydrolate 1	3	25	3:25 (1:8)
	T4 – *B. dracunculifolia* oil	2	23	2:23 (1:11.5)
	T5 – *Pinus elliottii* oil	2	23	2:23 (1:11.5)
	T6 – *S. granulosoleprosum* + *Ricinus communis* 1	3	25	3:25 (1:8)
	T7 – *S. granulosoleprosum* + *R. communis* 2	20	0	20:0 (Pure)
	T8 – Control (water)	0	20	0:20 (Pure)

(T8); *Pinus elliottii* hydrolate – 44.4% (T9); *P. elliottii* hydrolate – 10.7% (T10); *B. dracunculifolia* oil – 8% (T11); *P. elliottii* oil −8% (T12); *Solanum granulosoleprosum* + *Ricinus* 1–10.7% (T13); *S. granulosoleprosum* + *Ricinus* 2–100% (T14); water (T15) used as control treatment.

Once the pupae were sprayed, the dishes were capped and kept under controlled conditions (25 ± 2°C, 60% RH) until adult emergence. Three days after the last emergence of adult flies was observed, we dissected of uneclosed puparia in order to establish their condition. The number of emerged adults as well as the number uneclosed puparia (hollow or unhatched pupae) were both recorded for each treatment and fruit fly species.

Efficacy of the different biopesticides was assessed through the percentage of adult emergence (AE) for both Tephritidae species. The AE was calculated using the following formula: AE = *(n/N)*100*, where *n* = total number of emerged adults (males plus females) in the treatment, *N* = total number of pupae exposed to treatment.

Each treatment was performed in triplicate using different cohorts and repeated 10 times at each cohort (*n* = 30 per treatment).

Effect of ingestion on adult mortality

Thirty grams of pupae of each fly species were placed in a 500 mL plastic container in an acrylic cage (30 × 30 × 30 cm) until adult emergence (10 to 15 days after pupation). Two hours after eclosion, when adults were fully sclerotised and ready to be handled, flies were sorted both by species and sex with a plastic aspirator, and transferred in groups of 10 (5 ♂ + 5 ♀) into 1000 mL polyethylene terephthalate (PET) cages. Each cage was labelled, both for treatments and fruit fly species, and covered with cotton cloth to ensure ventilation and prevent escape of flies. A cotton wick soaked in a solution of sugar and hydrolysed protein at a 3:1 ratio (*w*/w) and a water dispenser of 5 mL with a mix of water + biopesticide (Table 1) was placed inside each cage. Adults were exposed to the biopesticide when drinking. Adult survival was checked every day over a 10-day period. Dead adults were removed daily to avoid further contamination. Ten replicates for each treatment were made. Each treatment was performed in triplicate using different cohorts and repeated 5 times at each cohort (totalising 150 adults flies per treatment). All cages were held in an environmentally controlled chamber at 26 ± 2°C, 60 ± 10% RH and 12:12 L:D photoperiod.

Statistical analyses

To meet parametric assumptions, percentage data were arcsine square root-transformed prior to analyses (Zar 1999); nevertheless, untransformed means (x ± SE) are shown in Table 2. To determine the effectiveness of biopesticides on the percentage of emergence as well as the percentage of survival of *Anastrepha fraterculus* and *Ceratitis capitata*, both data were subjected to a two-way general linear model type III error and level of significance of $\alpha = 0.05$. The fixed component of the model were treatments (i.e. biopesticides) and fruit fly species (*A. fraterculus* and *C. capitata*), and their interaction (Treatment * species). Mean comparisons were analysed and homogeneous groups were identified using Tukey's honestly significant difference (HSD) tests ($\alpha = 0.05$).

To determine whether different treatments influenced adult survival of *A. fraterculus* and *C. capitata*, longevity data were subjected to Kaplan–Meier survival analysis (SAS 2008, Goel *et al.* 2010). Finally, the log-rank tests were used to determine differences in survival between treatments ($\alpha = 0.05$; see Fig. 1).

Table 2 Percentage (mean ± SE) of adult fruit fly emergence for different biopesticides

Treatments	Percentage of adult emergence *Ceratitis capitata*	Percentage of adult emergence *Anastrepha fraterculus*
Pinus elliottii oil (T12)	0.00 ± 0.00 a	21.00 ± 5.42 b
Baccharis dracunculifolia oil (T11)	0.00 ± 0.00 a	15.00 ± 4.35 b
Solanum granulosoleprosum + *Ricinus communis* 2 (14)	64.00 ± 2.67 cd	57.00 ± 6.20 c
Spinosad (Flipper®) (T4)	73.00 ± 3.96 cde	74.00 ± 4.14 cde
P. elliottii hydrolate 2 (T10)	82.00 ± 1.70 de	81.00 ± 3.71 de
Bacillus thuringiensis – Delta endotoxin Baprom (T1)	81.00 ± 2.87 de	82.50 ± 3.35 de
B. dracunculifolia hydrolate 75% (T8)	83.00 ± 1.70 de	82.50 ± 2.01 de
Beauveria bassiana (T3)	81.50 ± 1.98 de	79.50 ± 1.57 de
B. thuringiensis var. Kurstaki Biospam (T2)	82.00 ± 1.70 de	81.00 ± 3.71 de
P. lliottii hydrolate 1 (T9)	80.00 ± 2.98 de	90.00 ± 1.83 e
B. dracunculifolia hydrolate 35% (T6)	86.00 ± 2.21 de	84.50 ± 2.73 de
Control (T15)	88.50 ± 4.02 de	87.50 ± 2.81 de
Spinosad (Tracer®) (T5)	8o.00 ± 2.11 de	94.50 ± 1.17 e
S. granulosoleprosum + *R. communis* 1 (T13)	83.00 ± 2.90 de	95.00 ± 1.50 e
B. dracunculifolia hydrolate 50% (T7)	93.00 ± 0.82 e	92.00 ± 1.11 e

Within columns figures followed by different lower case letters are statistically different at the 0.05 level.

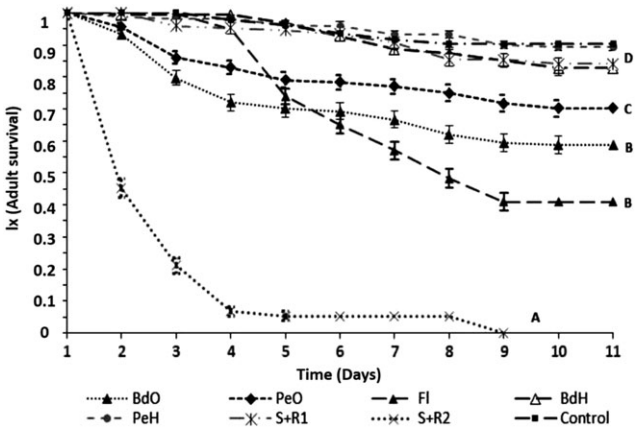

Fig. 1. Adults' survival of both tephritid species under eight different biopesticide treatments. Fl, spinosad (Flipper®); BdH, *Baccharis dracunculifolia* hydrolate; PeH, *Pinus elliottii* hydrolate; BdO, *Baccharis dracunculifolia* oil; PeO, *Pinus elliottii* oil; S + R1, *Solanum granulosum* + *Ricinus communis* 1; S + R2, *Solanum granulosum* + *Ricinus communis* 2; control. Means followed by the same letter are not significantly different ($P > 0.05$) (Log Rank (Mantel–Cox) test).

Statistical analysis of adult survival assays was performed with SPSS version 22.0 (IBM Statistics 22.0 Version, SPSS 2013). To determine the efficacy of products, STATISTICA, version 10.0 (StatSoft 2011) was used.

RESULTS

Effect of direct contact on pupae

There was a significant effect of biopesticide treatment ($F_{(14,\ 299)} = 181.36$, $P < 0.01$), and fruit fly species ($F_{(1,\ 299)} = 57.01$, $P < 0.01$), as well as their interaction ($F_{(14,\ 299)} = 24.59$, $P < 0.01$). Treatment with *Pinus elliottii* and *Baccharis dracunculifolia* oils resulted in the poorest adult emergence (Table 2). Treatment with the highest concentration of the *Solanum granulosoleprosum* + *Ricinus communis* extract (T14) resulted in a significant reduction of adult fruit fly emergence in comparison with the control treatment. All other topical biopesticide treatments on pupae had no effect on adult emergence when compared with the control treatment (Table 2). Overall, average adult emergence was higher for *Anastrepha fraterculus* (74.97 ± 1.15 a) than for *Ceratitis capitata* (70.80 ± 0.79 b). When treated with *P. elliottii* and *B. dracunculifolia* oils, no *C. capitata* adults emerged, but a small percentage of *A. fraterculus* did (15% and 21%, respectively). No significant differences were observed for the remaining treatments between species.

Effect of ingestion on the percentage of live fruit flies adults

There was a significant effect of biopesticide treatment on adult mortality ($F_{(7,159)} = 220.15$, $P <= 0.01$). There was no effect of species ($F_{(1,\ 159)} = 0.57$, $P = 0.45$) or the interaction of biopesticide and species on adult mortality ($F_{(7,\ 159)} = 0.11$, $P = 0.99$). Results from both species were therefore combined.

The pure *Solanum granulosoleprosum* + *Ricinus communis* (T7) caused the lowest percentage of living adults (0%), followed by spinosad (41%), *Baccharis dracunculifolia* and *Pinus elliottii* oils (58.67 and 70.33% respectively), *B. dracunculifolia* hydrolate (82.67%), and diluted *S. granulosoleprosum* + *R. communis* (T6) extract (84%) (Table 3). It was also observed that adults of both fly species were reluctant to approach water dispensers containing *B. dracunculifolia* or *P. elliottii* essential oils.

Survival of the adults

Kaplan–Meier analysis showed that adult survival was only affected by biopesticide treatment (treatment $\chi^2_{(7,\ 99)} = 666.43$, $P < 0.01$; species $\chi^2_{(1,\ 99)} = 0.13$, $P = 0.72$ and treatment * species interaction $\chi^2_{(7,\ 99)} = 3.293$, $P = 0.86$).

Table 3 Percentage of living fruit fly adults ($x \pm SE$) (*Ceratitis capitata* and *Anastrepha fraterculus* combined) after daily intake of different biopesticides

Treatment	Living adult (%)
Solanum granulosoleprosum + *Ricinus communis* 2 (T7)	0.00 ± 0.00 a
Spinosad (Flipper®) (T1)	41.00 ± 3.93 b
Baccharis dracunculifolia oil (T4)	58.67 ± 3.74 c
Pinus elliottii oil (T5)	70.33 ± 4.54 c
B. dracunculifolia hydrolate (T2)	82.67 ± 2.19 d
S. granulosoleprosum + *R. communis* 1 (T6)	84.00 ± 2.52 d
P. elliottii hydrolate (T3)	89.33 ± 1.59 de
Control (T8)	90.30 ± 2.12 e

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

Adults' survival for both hydrolates and diluted *Solanum granulosoleprosum* + *Ricinus communis* (T6) extract were similar and did not differ from the control. On the other hand, pure *S. granulosoleprosum* + *R. communis* (T7) Flipper, and both essentials oils (*Baccharis dracunculifolia* and *Pinus elliottii*) and the control significantly differed. The mean survival time (±SE) in days for adults was: 2.94 ± 0.094 S + R1 (*Solanum* + *Ricinus* 1), 8.21 ± 0.15 Fl (Flipper), 8.31 ± 0.20 BdO (*B. dracunculifolia* oil), 9.16 ± 0.18 PeO (*P. elliottii* oil), 10.25 ± 0.07 BdH (*B. dracunculifolia* hydrolate), 10.32 ± 0.10 S + R1 (*S. granulosoleprosum* + *R. communis 1*), 10.50 ± 0.09 control and 10.53 ± 0.09 PeH (*P. elliottii* hydrolate) treatments. The *Pinus* oil, *Baccharis* hydrolate, *Pinus* hydrolate, *Solanum* + *Ricinus* 1 and Control (water) showed survival rates from 88% to 95%. It is noteworthy that both species kept away from the drinking source in presence of oils, which could be considered a repellent effect and would be directly influencing the low mortality detected.

Chemicals

The identity and relative percent of the total compounds present in samples of *Baccharis dracunculifolia* and *Pinus elliottii* oils are shown in Tables 4 and 5, respectively. Identification of major peaks was achieved using NIST MS Search 2.0. Libraries.

DISCUSSION

Topical application of different biopesticides produced different effects on pupal mortality of *Anastrepha fraterculus* and *Ceratitis capitata*. Essential oils of *Baccharis dracunculifolia* and *Pinus elliottii* caused 100% mortality on *C. capitata* pupae and strongly suppressed adult eclosion of *A. fraterculus* in comparison with untreated controls (84% suppression of adult eclosion for *A. fraterculus* and 88.5% for *C. capitata*). These biopesticides contain, among other compounds, limonene and α-pinene and β-pinene, which are common components of other EOs used as biopesticides (Zibaee & Khorram 2015). Extracts of *Solanum granulosoleprosum* and *Ricinus communis* at a concentration of 8% also caused a significant reduction in adult eclosion (on both fly species) when compared with the control, but eclosion was nonetheless greater than 40%. According to Silva *et al.* (2005), only treatments that achieve a mortality of 40% or more could be considered as a promising product for control insect pests. All other tested products had no effect on pupal mortality. Toxicity on adults through ingestion was greatest for extracts of *S. granulosoleprosum* and *R. communis* at a concentration of 8%, with no survival of either species. This was followed by Spinosad (Flipper®) and *B. dracunculifolia* oil, although in these two cases, survival was above 40%. All other tested products had no effect on adult mortality. *R. communis*

Table 4 Retention times, relative percent area and compound identity of *Baccharis dracunculifolia* oil

Compound No	Retention time (s)	Relative percent area	Identification
1	8.90	1.16	Solvent derivates
2	11.15	5.36	α-pinene
3	12.57	0.53	Ni
4	12.81	22.69	β-pinene
5	13.07	1.92	β-myrcene
6	13.45	0.53	Ni
7	14.63	19.07	(±)-limonene
8	15.13	0.68	Ni
9	17.00	0.55	Ni
10	21.64	0.56	Ni
11	26.28	0.80	Ni
12	27.29	6.17	β-caryophyllene
13	27.80	1.72	(−)-alloaromadendrene
14	28.28	1.58	α-caryophyllene
15	28.70	1.01	γ-muurolene
16	28.95	4.16	(−)-germacrene D
17	29.20	1.70	(+)-ledene
18	29.35	7.80	γ-elemene
19	29.54	0.55	Ni
20	29.78	0.76	Ni
21	29.83	3.49	(+)-Δ-cadinene
22	30.78	8.08	nerolidol
23	31.52	5.90	(−)-spathulenol
24	31.72	2.04	Ni
25	32.00	1.19	ledol

Ni, not identified.

Table 5 Retention times, relative percent area and compound identity of *Pinus elliottii* oil

Compound *N*	Retention time (s)	Relative percent area	Identification
1	9.94	0.08	Ni
2	10.37	39.25	α-pinene
3	10.93	0.53	canfene
4	12.02	34.79	β-pinene
5	12.32	1.64	β-mircene
6	13.87	9.31	α-limonene
7	13.95	11.93	β-felandrene
8	17.99	0.17	Ni
9	19.83	0.65	α-terpineol
10	26.6	0.30	Ni
11	29.29	0.54	δ-cadinene
12	32.56	0.36	Ni
13	32.88	0.46	Ni

Ni, not identified.

plant extracts possess larvicidal properties providing an effective eco-friendly control for dipteran, coleopteran, lepidopteran and microbial pests (Upasani *et al.* 2003; Devanand & Rani 2008; Mandal 2010; Rampadarath & Puchooa 2016). On the other hand, high efficacy for the control of dipteran, coleopteran, homopterous, lepidopteran and mite pests were previously demonstrated for extracts of the Solanaceae family (Castillo-Sánchez *et al.* 2010).

EOs in several plant families have been found to negatively affect tephritid pests, and this can be exploited to design biorational management strategies. The EOs have been found to be toxic to adults, pupae, larvae and eggs of several species of fruit flies (Canale *et al.* 2013; Benelli *et al.* 2013; Vera *et al.* 2013; Buentello-Wong *et al.* 2016). Here, we focused on their effect on pupae and adults of the two most economically important species of fruit fly species in South America. These products can also act as repellants to females seeking egg-laying opportunities (Hidayat *et al.* 2013: Malheiro *et al.* 2015), can act as attractants to one or both sexes (Diongue *et al.* 2013), stimulate female oviposition activity (Ioannou *et al.* 2012) or even enhance male mating success (Shelly *et al.* 2004). In consequence, the fact that some compounds were not found to be toxic in our study does not mean that further testing on other interesting pest management properties should not happen. EOs have been reported to negatively affect insects through ingestion, contact and/or fumigation (Benelli *et al.* 2013). It would therefore be interesting to explore the effect of fumigation or exposure to vapour for *P. elliottii* and *B. dracunculifolia* EOs and hydrolates on different developmental stages of fruit flies. In some cases, a single EO can simultaneously produce various effects (Barud *et al.* 2014) and combined properties of such compounds could be exploited in designing efficient biorational management tools.

Our assays revealed a strong toxic effect of *B. dracunculifolia* and *P. elliottii* EO through contact on *C. capitata* and *A. fraterculus* pupae. These EOs could also be toxic to larvae, as this developmental stage has been found to be more susceptible to some EOs than pupae in laboratory assays (Papachristos *et al.* 2008). Toxic effects of EOs through topical application are easier to test on pupae and adults than on larvae in the laboratory because they do not involve handling of artificial diet or host fruit. But it is important to also test the effects of the product's toxicity on larvae and eggs after initial screening on pupae or adults and the potential field applications. Hydrolates of EOs are a cheaper product of the extraction process and in some cases, have been found to have some interesting properties for pest management purposes, such as ovicidal and anti-feeding effects (Rebolledo *et al.* 2012; Zekri *et al.* 2016). During our assays, these products were not found to be toxic to *C. capitata* or *A. fraterculus*. Nevertheless, we did not test for attraction, repellency or any physiological effect. We can therefore not rule out their potential use in pest management.

With respect to adult toxicity through ingestion, the best results were obtained with crude extracts of *S. granulosoleprosum* and *R. communis* at a concentration of 8%. Interestingly, these plant extracts were more effective than commercial formulations of insecticides recommended for fruit fly control in conjunction with feeding stimulants in Argentina (Flipper® (Spinosad). The use of botanical insecticides is therefore promising and could result in discovery of new and more effective and environmentally friendly tephritid control methods. Therefore, Neem (*Azadirachta indica*) has been reported as an effective biopesticide for several groups of arthropods (Girish & Shankara 2008).

We informally observed potential repellency of some products during ingestion assays that could be further exploited in pest management. The EOSs of several species of *Pinus* have high contents of limonene and α-pinene (Macchioni *et al.* 2003), which can also be found in plants in the genus *Baccharis* (Loayza *et al.* 1995) and have been found to be neurotoxic and repellent to insects (García *et al.* 2005). These two compounds and β-pinene were found in both *B. dracunculifolia* and *P. elliottii* oils in large proportions and are probably linked to the high mortality recorded during our assays. Interestingly, limonene is toxic to *C. capitata* larvae (Papachristos *et al.* 2009), but is also a component of male sexual pheromone of both *A. fraterculus* and *C. capitata* (Lima *et al.* 2001; Gonçalves *et al.* 2006), and has been found to be attractive to male and female *C. capitata* (Hernández-Sánchez *et al.* 2001).

The dual properties of some plant compounds could be exploited to design attract and kill strategies for pest management (Schumann *et al.* 2013), while other compounds or product combinations could be used in push (repel), pull (attract) and kill schemes (Cook *et al.* 2007). The underlying principle lies in exploiting insect response to plant compounds to manipulate pest movement in commercial orchards with aims at eliminating or reducing populations using highly specific and rapidly degradable pesticides. Exploring plant properties, chemical composition and insect response can therefore be a highly rewarding research avenue to develop sustainable pest management.

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