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Insecticidal effects of *Flourensia oolepis* Blake (Asteraceae) essential oil

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Abstract

Flourensia oolepis Blake (Asteraceae) essential oil had a complex chemical composition with τ-muurolene (6.14%), santolinetriene (6.22%), 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane (10.15%), δ-cadinene (10.27%) and γ-gurjunene (20.69%) comprising more than 50% of the oil. This oil had repellent and toxic effects on *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) adults, acting as a contact toxin. *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) adults showed behavioral sensibility to this oil.

Keywords: *Flourensia oolepis*; Essential oil; *Tribolium castaneum*; Toxicity; Repellency; *Myzus persicae*

1. Introduction

The extended use of broad-spectrum insecticides has resulted in the development of resistant insect populations (Bughio and Wilkins, 2004). Naturally occurring substances are an alternative to conventional pesticides (Plimmer, 1993) and plant essential oils, have traditionally been used to kill or repel insects (Isman, 2000).

Essential oils are effective against several insect species with varying potencies (Ho et al., 1996; Huang et al., 1999; Tunç et al., 2000; Zhu et al., 2001; Kostyukovsky et al., 2002; Garcia et al., 2005); acting as toxins, growth inhibitors, development disruptors, deterrents or repellents. Phytophagous insects use plant volatiles to recognize their host plants. Therefore, the use of essential oils as a non-host volatile emission to repel insect pests is a viable alternative for control (Mauchline et al., 2005).

Flourensia oolepis Blake (Asteraceae) is a common shrub growing in the central region of Argentina. Plant leaf surface is covered by a characteristic oil-fatty cuticle that could act as a defensive barrier. Cuticular waxes are an

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important interface in trophic interactions and their substances function as allomones deterring oviposition and feeding by herbivores (see Müller and Riederer, 2005). Resin and essential oil components of other *Flourensia* spp. species have been reported as having insect antifeedant (Faini et al., 1997), phytotoxic (Mata et al., 2003), antifungal, antialgal, and antitermite properties (Tellez et al., 2001). However, the defensive properties of *F. oolepis* oil are not known.

As part of our ongoing study of botanical insecticides from plants growing in semiarid central-western area of Argentina, we have studied the composition and biological effect of *F. oolepis* essential oil on the following insect species with different feeding adaptations. The generalist *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), a world-wide pest of stored grains which is sensitive to some essential oils (Liu and Ho, 1999; Padin et al., 2000), *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), one of the major pest of solanaceous crops in America and Europe that has developed resistance to synthetic insecticides; the aphids *Rhopalosiphum padi* L. (Homoptera: Aphididae) with *Prunus padus* L. as their primary host and members of the Poaceae as secondary ones (Huggett et al., 1999) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae), a world-wide polyphagous aphid which feeds on more than 400 plant species, including many agricultural crops (Margaritopoulos et al., 2004).

2. Material and methods

2.1. Plant material

Aerial parts of *F. oolepis* Blake (Asteraceae) were collected in February 2001, at El Volcan, San Luis, Argentina. A voucher sample was deposited at the Herbarium of the Universidad Nacional de San Luis, Argentina (2329-Del Vitto).

2.2. Extraction of essential oil

Fresh aerial parts (6.050 g) were cut into small pieces and subjected to steam-distillation at 96 °C for 3 h using a Clevenger-type apparatus; the oil obtained (0.803 g kg^{-1}) was dried over anhydrous sodium sulfate.

2.3. Identification of components

The essential oil composition was determined by GC–MS. Retention times and mass spectral data were compared with the MS instrument library and NIST library. Relative percentages of the major components were calculated by integrating the registered peaks. GC–MS experiments were performed on an ion trap GCQ-Plus (Finnigan, ThermoQuest, Austin, TX, USA) instrument with MS–MS program using a silica capillary column Rtx®-5MS (30 m × 0.25 mm ID, 0.25 μm). The carrier gas was helium (40 cms^{-1}). The port temperature was 200 °C in splitless mode with 1.0 μl injection volume. The initial temperature was maintained at 40 °C for 2 min, and was then increased to 210 °C at 2 °C min^{-1}, and maintained at this temperature up to 120 min.

For the analysis of low resolution MS, the ion trap mass detector was set in full scan mode from *m*/*z* 50 to *m*/*z* 450. For product analysis (CID), the precursor was selected using tandem mass spectrometry (MS/MS) scan standard function, with 0.5 Da peak-width for the parent ion and dynamically programmed scans, as described previously (Ardanáz et al., 1991).

2.4. Insect bioassays

T. castaneum colony was reared on a mixture of flour, yeast and starch (3:3:1) at 25 ± 1 °C, 65% relative humidity, and a 16:8 (L:D) photoperiod in a growth chamber. *L. decemlineata*, *M. persicae* and *R. padi* were reared on their respective host plants (*Solanum tuberosum* L., *Capsicum anuum* L. and *Hordeum vulgare* L.) and maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 (L:D) in a growth chamber.

2.4.1. Repellency against T. castaneum adults

Choice bioassays consisted of two joined 125 ml Erlenmeyer flasks as described previously (Garcia et al., 2005). Nine *n*-hexane solutions with increasing concentrations of essential oil were prepared to give final concentrations ranging between 0.0 and 0.750 mg/cm^2. Twenty adults of *T. castaneum*, randomly selected, were used for each

treatment and replicated three times. Bioassays were conducted in complete darkness at 25 ± 1 °C, and 65% humidity. After 30, 60, 90, 150 and 210 min, the Response Index (RI) (Phillips et al., 1993) was calculated as RI = ($T - C$/Tot)100, for which T is the number responding to the treatment; C is the number responding to the control and Tot is the total number of insects released. To determine significant differences among treatments and time of exposure, data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons tests at $P < 0.05$. ED_{50} values were determined from linear regression analysis (RI on log dose).

2.4.2. Toxicity against T. castaneum adults

Twenty *T. castaneum* adults randomly selected were placed in treated Erlenmeyer flasks as previously described (Garcia et al., 2005) and kept at 25 ± 1 °C with a 16:8 (L:D) photoperiod. Each treatment was repeated three times. Insect mortality was recorded after 24, 48 and 72 h. Percent insect mortality was corrected according to Abbott (1925). To determine significant differences among treatments and time of exposure, data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons tests at $P < 0.05$.

2.4.3. Feeding inhibition against L. decemlineata adults

These experiments were conducted with *L. decemlineata* adults. Percent feeding inhibition (%FI) was calculated as described by Reina et al. (2001). Treatment with an FI > 70% were tested in a dose–response experiment to calculate their ED_{50} values which were determined from linear regression analysis (%FI on log dose).

2.4.4. Settling inhibition of M. persicae and R. padi adults

These experiments were conducted with apterous adults of both species. Percent settling inhibition (%SI) was calculated as described by Gutiérrez et al. (1997). Difference between control and treatment was determined using the Mann–Whitney test. Treatment with an SI > 70% were tested in a dose–response experiment to calculate their EC_{50} values, which were determined from linear regression analysis (%SI on log dose).

3. Results and discussion

Table 1 shows the chemical composition of *F. oolepis* essential oil. A total of 38 compounds were identified (93.04%). τ-Muurolene (6.14%), santolinetriene (6.22%), 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane (10.15%), δ-cadinene (10.27%) and γ-gurjunene (20.69%) accounted for more than 50% of the oil. Overall, this oil was rich in sesquiterpene hydrocarbons (47.4%), oxygenated sesquiterpenes (23.7%), and monoterpene hydrocarbons (21.0%), with the oxygenated monoterpenes (5.3%) and triterpene hydrocarbons (2.6%) being the least abundant terpene groups.

Sesquiterpenes and flavonoids have been isolated from *F. oolepis* (Guerreiro et al., 1979) and the essential oil composition reported for plants collected in Cordoba Province with τ-cadinol (10.5%), β-selinene (9.8%), linalool (8.2%) and β-eudesmol being the major components (Priotti et al., 1997). However, as reported here, the oil from the plants collected in San Luis Province had a very different composition and a minor ratio of oxygenated terpenes (29.0% vs. 45.5%), suggesting a major volatility for this oil and the presence of different chemotypes.

Similarly, the essential oil of *Flourensia cernua*, a species abundant in northern to central deserts of Mexico with an oil composition very different from that of *F. oolepis* (myrcene/3-δ-carene/limonene) (Tellez et al., 2001), has a great degree of variability in leaf surface mono and sesquiterpenoid concentration in different plants (Estell et al., 1994).

Table 2 shows the behavioral effects of *F. oolepis* essential oil on *T. castaneum* adults. This oil had a dose-dependent repellent effect (30 min: $K - W = 32.72$, df = 8, $P < 0.001$; 60 min: $K - W = 32.82$, df = 8, $P < 0.001$; 90 min: $K - W = 32.39$, df = 8, $P < 0.001$; 150 min: $K - W = 32.92$, df = 8, $P < 0.001$; 210 min: $K - W = 32.69$, df = 8, $P < 0.001$) regardless of the exposure time ($P > 0.05$). *T. castaneum* adults responded in a similar way to an oil dose range of 0.192–0.750 mg/cm^2. The insects showed a clear orientation choice between *F. oolepis* volatiles and the control, indicating that *T. castaneum* adults are able to detect the oil through olfaction. There was no significant variation with time indicating that *T. castaneum* olfactory receptors responded to each oil concentration within 30 min of exposure, maintaining the same response to each concentration with time up to 210 min. The essential oil of *F. oolepis* had moderate dose-dependent toxic effects against *T. castaneum* adults (24 h: $K - W = 24.26$, df = 8, $P = 0.002$; 48 h: $K - W = 24.16$, df = 8, $P = 0.002$ and 72 h: $K - W = 23.75$, df = 8, $P = 0.002$), showing a small, not significant ($P > 0.05$), increase in mortality with time for the two highest doses tested (Table 3), suggesting that significant

Table 1
Chemical composition of *F. oolepis* essential oil

Components[a]	RI[b]	Abundance (%)
Santolinetriene	8.01	6.22
α-Pinene	8.74	1.89
β-Pinene	10	0.25
3-Carene	10.49	0.34
α-Thujene	10.89	0.04
Limonene	11.65	0.88
(+)-2,6,6-Trimethyl-bicyclo[3.1.1]-2-heptene	11.94	0.30
Ocimene	12.31	2.56
μ-Terpineol	16.27	1.17
α-Terpineol	16.65	0.15
α-Cubebene	21.06	0.43
Copaene	21.79	1.47
2-Methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane	23.02	10.15
Alloaromadendrene	23.48	1.83
α-Muurolene	23.77	0.96
α-Caryophyllene	23.87	0.62
Aromadendren	24.06	2.55
α-Longipinene	24.45	2.70
γ-Gurjunene	25.02	20.69
β-Muurolene	25.39	3.51
δ-Cadinene	25.62	10.27
1,2,3,4,4α,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	25.82	0.49
τ-Elemene	26.48	0.77
(−)-Spathulenol	26.99	1.02
Caryophylene oxide	27.14	2.88
Z-α-*trans*-bergamotol	27.31	1.37
Cubenol	27.64	1.53
τ-Cadinene	28.14	1.08
τ-Muurolene	28.45	6.14
Ledene oxide-(II)	28.68	0.31
Eremophilene	28.77	2.17
Dehydro-aromadendrene	29.19	0.22
Guajazulene	29.23	0.16
2-(4α,8-dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	30.78	0.17
8-Cedren-13-ol	31.01	0.22
Selinane	32.28	0.78
1-[6-hydroxy-2-(1-methylethenyl)-7-benzofuranyl]-ethanone	33.2	1.28
Squalene	39.26	3.48

[a] Identified components: 93.04%.
[b] Retention index relative to C_8–C_{23} *n*-alkanes on Supelcowax™ 10 column.

time-dependent toxic effects could be expected for higher doses. Insects exposed to oil doses between 0.190 and 0.750 mg/cm^2 suffered tremors, convulsions and lack of mechanical coordination, indicating a neurotoxic action.

Similarly, the behavioral effects of *Baccharis salicifolia* (Ruiz & Pavon) Pers essential oil on *T. castaneum* were not time-dependent, while its toxicity increased with time (Garcia et al., 2005).

Essential oils are highly volatile mixtures with repellent and insecticidal properties, including contact and/or fumigant activities against stored product insects such as *T. castaneum* (Saraç and Tunç, 1995a,b; Huang et al., 1997; Shaaya et al., 1997; Garcia et al., 2005). However, the volatility of essential oils can make it difficult to discriminate between their fumigant and contact toxicity. In our experiment, the essential oil vapors were not toxic to *T. castaneum* (data not shown), indicating that *F. oolepis* essential oil is a contact insecticide.

F. oolepis essential oil produced an 83% feeding reduction in the case of *L. decemlineata* adults at a dose of 100 μg/cm^2. There was a significant correlation between %FI and concentration ($P < 0.05$; $R^2 = 99.1$), with a moderate effective dose (ED_{50}) of 19 μg/cm^2 (4.8–76.8, 95% confidence limits). This insect showed behavioral responses to natural blends of volatiles emitted by plants (Dickens, 2000, 2002), including essential oils (Panasiuk, 1984).

Table 2
Response Index data of *F. oolepis* essential oil on *T. castaneum* adults at different times (min), in a two-choice bioassay

[mg/cm^2]	Time-course of the Response Index (±SD)				
	30 min	60 min	90 min	150 min	210 min
0.00	3.00 (7.7)	3.00 (7.7)	0.00 (6.6)	3.00 (8.8)	−3.00 (7.7)
0.006	−3.00 (5.7)	−10.00 (10.0)	0.00 (10.0)	−3.00 (5.7)	−3.00 (5.7)
0.012	−43.00 (5.7)	−43.00 (5.7)	−36.00 (11.5)	−26.00 (5.7)	−26.00 (5.7)
0.024	−43.00 (5.7)	−43.00 (5.7)	−46.00 (5.7)	−43.00 (5.7)	−46.00 (5.7)
0.048	−53.00 (5.7)	−53.00 (5.7)	−53.00 (5.7)	−43.00 (5.7)	−43.00 (5.7)
0.096	−80.00 (10.0)	−77.00 (5.7)	−83.00 (5.7)	−90.00 (0.0)	−86.00 (5.7)
0.192	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*
0.500	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*
0.750	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*	−100.00 (0.0)*
ED_{50}	0.031	0.029	0.033	0.037	0.037
(95% CL)	(0.017–0.055)	(0.016–0.049)	(0.018–0.058)	(0.020–0.066)	(0.021–0.064)

Each data point represents the mean of three replicates with 20 adults each ($n = 60$). Means within a column followed with * are significantly different from the control at $P < 0.05$ (Kruskal–Wallis test followed by Dunn's multiple comparison test).
Effective dose (ED_{50}) and 95% confidence limits (Lower, Upper).

F. oolepis essential oil affected the settling behavior of the polyphagous aphid *M. persicae* in a dose dependent manner, while *R. padi* did not respond to this oil (Table 4). This oil had potency levels similar to farnesol, an alarm pheromone precursor (Gutiérrez et al., 1997). *M. persicae* has shown multiple insecticide resistance mechanisms due to the continuous use of insecticides (Mazzoni and Cravedi, 2002), therefore *F. oolepis* essential oil could be a possible control agent of this aphid as part of an integrated pest management strategy.

The dose–response behavioral and/or toxic effects observed for *F. oolepis* oil on the insect species tested here (*T. castaneum*, *L. decemlineata* and *M. persicae*) suggest that these actions could be attributed to the oil's main components. α-Gurjunene was a repellent to the sweet potato weevil *Cylas formicarius elegantalus* Summers (Coleoptera: Curculionidae) and has been related to the toxicity of *Tagetes minuta* root exudates to aquatic macroinvertebrates (Kumar et al., 2000; Wang and Kays, 2002). Bycyclic acetals, such as 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane, play an important role in chemical communication systems among many insect species (Francke and Schroder, 1999). Cadinane-type sesquiterpenes provide constitutive and inducible protection against pests and plant diseases (Townsend et al., 2005) and muurolene has been related to the host selection process of *Thaumetopoea pityocampa* females (Zhang et al., 2003). Hexane and ether volatile extracts of *F. cernua* L. with limonene/myrcene/3δ-carene and germacreneD/β-caryophyllene being the major components, respectively, showed a high degree of antitermite activity, suggesting the presence of more than one active compound (Tellez et al., 2001). Essential oil components can have synergistic biological effects. For example, *Salvia lavanduleifolia* Vahl oil inhibits the enzyme acetylcholinesterase through a complex interaction between its components, including both

Table 3
Mortality data of *F. oolepis* essential oil on *T. castaneum* adults at different times (hours), in a contact toxicity bioassay

[mg/cm^2]	Percent mortality (±SD)		
	24 h	48 h	72 h
0.00	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
0.006	1.00 (2.8)	5.00 (5.0)	5.00 (5.0)
0.012	5.00 (5.0)	5.00 (5.0)	5.00 (5.0)
0.024	6.00 (2.8)	10.00 (0.0)	11.00 (2.8)
0.048	10.00 (0.0)	11.00 (2.8)	15.00 (5.5)
0.096	11.00 (2.8)	16.00 (2.8)	18.00 (2.8)
0.192	13.00 (2.8)	15.00 (5.0)	15.00 (0.0)
0.500	21.00 (2.8)	23.00 (2.8)	26.00 (2.8)
0.750	25.00 (5.0)*	28.00 (2.8)*	33.00 (2.8)*

Each data point represents the mean of three replicates with 20 adults each ($n = 60$). Means within a column followed with * are significantly different from the control at $P < 0.05$ (Kruskal–Wallis test followed by Dunn's multiple comparison test).

Table 4
Effective antifeedant dose (ED_{50}) and 95% confidence limits (Lower, Upper) of *F. oolepis* essential oil on *R. padi* and *M. persicae* apterous adults

Aphid	Dose (μg/cm^2)	*%C*	*%T*	*P*	%SI[a]	ED_{50} (μg/cm^2)
R. padi	100	51.33	48.66	>0.05	11. 41	–
M. persicae	4	49.20	50.79	>0.05	28.23	
	20	55.29	44.70	>0.05	36.82	
	50	80.96	19.03	<0.001	68.90	
	100	84.84	15.15	< 0.001	77.63	21.03(5.34–82.72)

$P < 0.05$ (Wilcoxon Signed Rank Paired test).
[a] %SI = [1 − (%T/%C)]100, where *%T* = percent aphids on treated disk and *%C* = percent aphids on control disk.

synergistic and antagonistic interactions (Savelev et al., 2003). This study shows that high 1,8-cineole and low camphor contents in the oil may increase its anticholinesterase activity. Therefore, further research is needed in order to understand the role that the major components of *F. oolepis* oil play in its insecticidal effects.

In summary, *F. oolepis* essential oil rich in the sesquiterpene hydrocarbons 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane and δ-cadinene as well as in the oxygenated sesquiterpene γ-gurjunene was active against two polyphagous insects with divergent feeding adaptations (*T. castaneum* and *M. persicae*). This oil had repellent and contact toxic effects on *T. castaneum* adults, and affected the settling and feeding behavior of *M. persicae*. The feeding behavior of *L. decemlineata* adults was moderately affected by this oil. Therefore, *F. oolepis* essential oil could be used to control *T. castaneum* infestations due to its toxic and repellent action. Since little is known about *F. oolepis* chemotypes, plant-to-plant variability should be studied concerning oil composition and insecticidal effect to detect chemotypes suitable for field adaptation and production.

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