Chemical Composition and Fumigant Toxicity of the Essential Oils From 16 Species of *Eucalyptus* Against *Haematobia irritans* (Diptera: Muscidae) Adults

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ABSTRACT Oils extracted from various species of *Eucalyptus* (*Eucalyptus badjensis* Beuzev & Welch, *Eucalyptus badjensis* × *Eucalyptus nitens*, *Eucalyptus benthamii* variety *dorrigoensis* Maiden & Cambage, *Eucalyptus botryoides* Smith, *Eucalyptus dalrympleana* Maiden, *Eucalyptus fastigata* Deane & Maiden, *Eucalyptus nobilis* L.A.S. Johnson & K. D. Hill, *Eucalyptus polybractea* R. Baker, *Eucalyptus radiata* ssp. *radiata* Sieber ex Spreng, *Eucalyptus resinifera* Smith, *Eucalyptus robertsonii* Blakely, *Eucalyptus rubida* Deane & Maiden, *Eucalyptus smithii* R. Baker, *Eucalyptus elata* Dehnh, *Eucalyptus fraxinoides* Deane & Maiden, *E. obliqua* L'Hér) were obtained by hydrodistillation. The chemical composition of essential oils was determined by gas chromatography coupled to mass spectrometry. Essential oils were mainly composed of 1,8-cineole, α-pinene, α-terpineol, 4-terpineol, and *p*-cymene. Vapors from these essential oils and their major components were found to be toxic to *Haematobia irritans* (L.) (Diptera: Muscidae) adults. An aliquot of each oil was placed in a cylindrical test chamber, and the number of knocked down flies was recorded as a function of time. Knockdown time 50% was then calculated. Results showed that essential oil of *E. polybractea* had the highest knockdown activity of 3.44 min. A correlation was observed between the content of 1,8-cineole in the *Eucalyptus* essential oils and the corresponding toxic effect.

KEY WORDS horn flies, control, knockdown, *Eucalyptus*, *Haematobia irritans*

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is one of the most important external parasites of beef and dairy cattle. Now distributed worldwide, this arthropod reached Europe by the migration of livestock from Asia (Kuramochi 2000). In the New World, the horn fly was reported first in 1887 by Riley in the United States (Riley 1887) and later dispersed into Canada and Central America (Vogelsang and De Armas 1940). In 1957, it was reported in Brazil and in 1991 was found in Argentina (Luzuriaga et al. 1991).

Adult horn flies spend most of their time on the back of cattle, only leaving their host to lay eggs in fresh dung pats, returning quickly to their host to blood feed (Thomsen 1938, Heine 1987, Hillerton 1988). Although flies prefer to stay on the host, they have the capacity to spread to other herds; the ability of horn flies to fly several kilometers to find a host has been demonstrated in a number of investigations (Jensen et al. 2004).

Heavy infestations can irritate and stress livestock, reducing beef and milk production (Kunz et al. 1984, Schreiber et al. 1987). The leather industry also is strongly affected because the presence of these flies produces irreversible damage to the raw material due of its bite (Guglielmone et al. 1999).

The control of horn flies includes chemical formulations such as pour-on or spot-on treatments and ear tags, which provide excellent residual control (Campbell and Thomas 1992). Chemicals commonly used include pyrethroids, mainly cypermethrin (Barros et al. 2007). However, pyrethroids have drawbacks because horn flies have become resistant to these compounds (McKenzie and Byford 1993, Guglielmone et al. 2001b). Due to the resistance of pyrethroids, organophosphates such as chlorpyrifos, diazinon, and ethion are being used (Guglielmone et al. 2001a).

An alternative to conventional insecticides is using products with botanical origins. Plants produce chemicals as defense mechanisms against the attack of microorganisms and predators. The essential oils are volatile substances that are found in a wide variety of plant species (Weinzieri et al. 1994, Weinzieri 2000) and are natural candidates for the development of new products for controlling insect pests (Lucia et al. 2008,

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Table 1. Origin and CSIRO identification number. for 16 species of *Eucalyptus* analyzed

Species	Identification no. CSIRO[a]	Place of origin
E. badjensis Beuzev & Welch	592/44	Badja State Forest, New South Wales (Australia) (BSF 30)
E. badjensis × *E. nitens*	92/44	Badja State Forest, New South Wales (Australia) (BSF 31)
E. benthamii var. *dorrigoensis* Maiden & Cambage	18763	Clouds Ck. Armidale, New South Wales (Australia)
E. botryoides Smith	KS 92/42	Mahongany Road via Nowa Victoria (Australia)
E. dalrympleana Maiden	KS 95/16	Kosciusko National Park, New South Wales (Australia)
E. fastigata Deane & Maiden	15127	30 km SE of Braidwood, New South Wales (Australia)
E. nobilis L.A.S. Johnson & K.D.Hill	19452	Styx River State Forest, New South Wales (Australia)
E. polybractea R. Baker	17433	Whipstick Forest, Victoria (Australia)
E. radiata ssp. *radiata* Sieber ex Spreng	18381	Nerrigundah, New South Wales (Australia)
E. resinifera Smith	13953	Nowra, New South Wales (Australia)
E. robertsonii Blakely	KS 95/16	Kosciusko National Park, New South Wales (Australia)
E. rubida Deane & Maiden	KS 95/16	Kosciusko National Park, New South Wales (Australia)
E. smithii R. Baker	18284	Tallaganda State Forest, New South Wales (Australia)
E. elata Dehnh	15103	Nepean River, New South Wales (Australia)
E. fraxinoides Deane & Maiden	88/26	Bald Peak to Pikes Saddle Tallaganda S.F., New South Wales (Australia)
E. obliqua L'Hér	93/15	Otways Messmate, Victoria (Australia)

[a] CSIRO, Commonwealth Scientific and Industrial Research Organization.

Picollo et al. 2008). The active components of essential oils are isoprenoid compounds, mainly mono- and sesquiterpenes, that are responsible for the odor of aromatic plants (Franzios et al. 1997). The insecticide mode of action of terpenoids is still not fully understood; however, the onset of toxicity is usually rapid (Enam 2001), indicating a neurotoxic mode of action (Isman 2006). Because the terpenoid components of these essential oils are volatile, their principal route to enter into an insect's body is through the respiratory system (Mill 1985).

The genus *Eucalyptus*, native from Australia and other surrounding Pacific islands, comprises of >800 species (Coppen 2002) and they have a variety of aromatic oils with characteristic odors. The oil glands of the plant are located inside the leaves, well below the epidermal cuticle and other cells forming the surface layers of the foliage. The simplicity of extracting essential oils from aromatic plants by using steam distillation (Denny 2002) and their diverse chemical compositions make them potential sources of natural pesticides, and they have attracted attention among researchers (Singh and Upadhyay 1993).

In this study, we analyzed the chemical composition of the essential oils obtained from 16 species of *Eucalyptus* and examined the knockdown activity of these oils and their major components against the horn fly.

Materials and Methods

Biological Material. Horn fly adults were collected from 20 Holstein cattle that were never exposed to insecticides. The dairy was located in Vicente Casares, the province of Buenos Aires, Argentina (35° 1′ S, 58° 34′ W). Horn flies were collected directly from the back of the animal using an aerial net and were placed in cubic acrylic cages 30 by 30 by 30 cm with two 6- by 19-cm screen grids. Flies were immediately fed through the mesh screen by placing a five by 18-cm piece of cotton soaked with fresh bovine blood (Frigorífico Velsud, Monte Grande, Buenos Aires, Argentina) on each screen grid. After the flies were introduced into the cage, the opening was closed with a piece of Lycra. Inside the cage, two round disposable polypropylene petri dishes containing cotton soaked with tap water were introduced. After horn fly collection, the cages were immediately transported to the research center, and the flies were maintained only with water at 25 ± 1°C and a photoperiod of 6:6 (L:D) h for at least 12 h before conducting bioassays.

Plant Material. The essential oils from 16 *Eucalyptus* species were obtained by hydrodistillation. Leaves were randomly selected from trees from a 10-yr old plantation located at the Agricultural Experiment Station, National Institute of Agricultural Technology Concepción del Uruguay, the province of Entre Ríos, Argentina (32° 29′S; 58° 20′W). See Table 1 for a list of *Eucalyptus* species collected.

To harvest the leaves of each *Eucalyptus* species, an aerial work platform was used (Hydro-Grubert BL 12-T; Hydro-Grubert, Córdoba, Argentina) to rise to the level where fully developed leafy branches were available. Branches were cut with a handsaw and 5 kg of leaves were placed in bags and kept in a shed at room temperature (20°C) until the following day, when the bags were sent to the laboratory (Centro de Investigaciones de Plagas e Insecticidas, Buenos Aires, Argentina). After a period of 3–5 d of drying at 20°C, leaves were processed to obtain essential oils.

Essential Oil Extraction. For each *Eucalyptus* species, at least 445 g of fresh leaf material was used to extract essential oils using hydrodistillation in a modified Clevenger-type apparatus (Figmay S.A., Córdoba, Argentina). The essential oil extract was then cooled, separated from the cohobated water, dehydrated with anhydrous sodium sulfate, and stored at –4°C until used.

Chemicals. The following chemicals were purchased from Sigma-Aldrich (Buenos Aires, Argentina) and used as chromatographic standards: *p*-cymene (99%), 1,8-cineole (99%), α-pinene (97%), α-phellandrene (95%), limonene (97%), γ-terpinene (97%), 4-terpineol (>96%), α-terpineol (99%), and β-eudes-

mol (90%); *p*-cymene, 1,8-cineole, and α-pinene were also used for the bioassay. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) 96.7%, used as a positive control, was provided by Chemotecnica S.A (Buenos Aires, Argentina).

Essential Oil Analysis. The chemical composition of *Eucalyptus* spp. essential oils was determined using a GCMS-QM 5050A instrument (Shimadzu, Kyoto, Japan) in the electron impact (70-eV) mode. Samples were analyzed on an apolar GC capillary column (0.25 mm i.d. × 30 m; 0.25-μm coating thickness, DB-5; J&W Scientific, Folsum, CA). The GC column temperature was programmed from 60 to 246°C at 3°C/min, and the injector was held at 220°C. Helium was used as the carrier gas, at a 1.02 ml/min caudal; the split ratio was 1:20 and 0.4 μl of a hexane solution of *Eucalyptus* essential oil or reference compounds (1 mg/ml) was injected. Essential oil components were identified for each sample by comparing the mass spectra to the available National Institute of Standards and Technology (NIST) or Wiley mass spectral library. Confirmation was performed by comparing GC retention times with chromatographic standards. Quantification of essential oil components (expressed in relative percentage per total area of chromatogram) was carried out by peak area normalization measurements (Adams 2007).

Fumigant Activity Bioassay. The bioassay was conducted following the method described for KT_{50} determination in mosquitoes (Lucia et al. 2009). A coverglass (18 by 18 mm long, 0.17 mm in thickness, Sailing Boat, Jiangsu, China) with 3 μl of pure essential oil was placed in an experimental unit that consisted of a transparent acrylic tube (12.7 cm in long by 5.1 cm in diameter, BioQuip Products, Rancho Dominguez, CA) covered at one end by a disc of neoprene and at the other by a metal wire mesh (mesh size, 0.86 mm) (Fig. 1).

The acrylic chamber was warmed in an incubator for 10 min at 26–28°C to saturate the container with oil vapors. Batches of 13–15 adult flies were collected with a mouth aspirator and introduced to the tube through the neoprene disc located on the upper side. Horn flies were exposed to the essential oil vapors without entering into direct contact with the coverglass. There were four replicates for each essential oil tested.

The number of flies knocked down was recorded every minute for ≈20–30 min. Insects lying on the filter paper, unable to fly, were considered knocked down. Dichlorvos was used as a positive control. As negative control, experimental units with just a coverslip were used. KT_{50} values in all the cases were >1 h. The total number of dead flies in each tube was counted after each bioassay.

Statistical Analysis. Knockdown data for each essential oil or component and for dichlorvos were subjected to probit analysis (Litchfield and Wilcoxon 1949). KT_{50} values were obtained using POLO PC 2.0 software. KT_{50} values were considered significantly different when the 95% confident limits (CL) did not overlap. The relationship between the fumigant activity [$KT_{50(min)}$] of essential oils and their content in major terpenoids was analyzed by simple and multiple regression using Statistical Graphics (Warrenton, VA) SGWIN software.

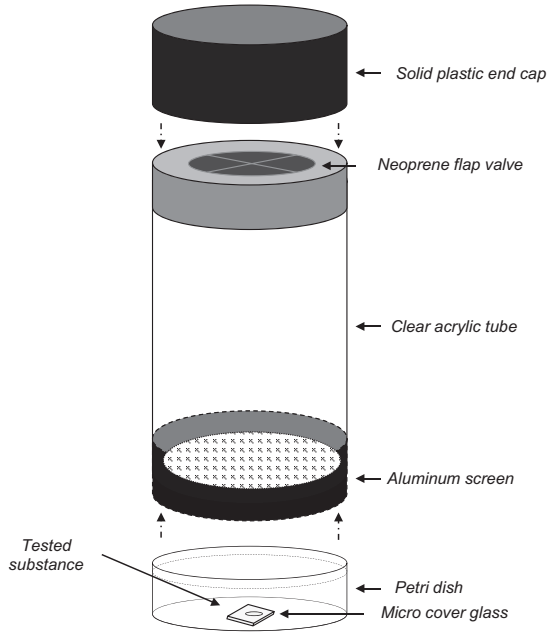

Fig. 1. Test device used to evaluate the fumigant activity of essential oils (Lucia et al. 2009) against *H. irritans*.

Results and Discussion

The main essential oil components of 11 *Eucalyptus* species: *Eucalyptus badjensis*, *E. badjensis* × *Eucalyptus nitens*, *Eucalyptus darlympleana*, *Eucalyptus nobilis*, *Eucalyptus polybractea*, *Eucalyptus radiata*, *Eucalyptus robertsonii*, *Eucalyptus resinifera*, *Eucalyptus dorrigoensis*, *Eucalyptus smithii*, and *Eucalyptus rubida* was 1,8-cineole, whereas the main component of *Eucalyptus elata*, *Eucalyptus fastigata*, *Eucalyptus fraxinoides*, and *Eucalyptus obliqua* was *p*-cymene. The main components of the *E. botryoides* oil were *p*-cymene, β-eudesmol, and 1,8-cineole (Table 2).

Essential oil insecticidal activity is species-dependent because their terpenoid composition influences insect toxicity (Isman 1999, Enam 2001). The KT_{50} values of the essential oils from different *Eucalyptus* plants varied according to the chemical composition of the oil (Table 3). The most active essential oil was obtained from *E. polybractea*, with a KT_{50} value of 3.44 min. Studying the chemical composition of this species showed that it contained 85.01% 1,8-cineole (Table 2). Therefore, the fumigant activity of *E. polybractea* essential oil could be due to the presence of a high amount of 1,8-cineole.

The fumigant activity tests for the individual pure oil components showed the following order of knockdown activity, obtained by comparing the $KT_{50(min)}$ values of each oil component: 1,8-cineole > *p*-cymene > α-pinene (Table 4).

Table 2. Chemical composition of essential oils from 16 species of *Eucalyptus*, expressed as relative percentage of the total area of the chromatogram

Oil constituent[a]	Plant species[b]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
α-Pinene	5.09	4.51	4.42	2.36	2.67	0.64	0.68	1.22	12.89	0.32	0.20	2.80	10.02	1.64	1.41	4.61
α-Phellandrene			0.27		0.78	15.98	3.18	3.12	1.21	2.04		0.19		2.46		
p-Cymene	2.36	1.24	19.91	5.61	1.50	14.77	37.56	35.46	18.21	25.43	4.12	0.70	11.95	2.77		
Limonene	6.00	5.91	3.10	4.80	5.19			0.32	2.39	0.30	1.00	7.32	3.60	4.16	4.07	5.88
β-Phellandrene						14.49	9.22	8.79		3.17						
1,8-Cineole	71.73	82.75	13.26	80.31	74.73	2.94	14.69	13.43	30.43	7.87	85.01	68.36	58.60	62.01	82.53	78.49
γ-Terpinene	0.6		5.00	0.90		0.56			7.03		0.33	0.53	2.28	1.59	0.74	0.65
cis-*p*-Menth-2-en-1-ol						12.21		4.73		11.97		0.09		0.70		
trans-*p*-Menth-2-en-1-ol						8.64	3.91	3.44		8.67		0.06		0.52		
4-Terpineol	0.45	0.47	0.40	0.49	0.46	4.83	2.76	2.72	1.72	1.51	1.48	1.84	1.30	5.99	0.42	0.60
α-Terpineol	1.26	0.96	3.90	1.46	0.87	1.51	2.47	2.28	0.55	1.05	0.70	12.44	4.22	8.60	1.73	2.48
Piperitone						0.44	4.03	4.10		23.22				0.63		
Viridiflorol	0.71	0.51	0.84	0.62	7.37		0.58		11.32		0.59	0.23	0.74			
β-Eudesmol	7.78	0.77				1.70										5.46
α-Eudesmol			14.97	0.58			5.29	7.92								
Other compounds[c]	4.02	2.88	33.93	2.87	6.43	21.29	13.93	12.47	14.25	14.45	6.57	5.44	7.29	8.93	9.1	1.83

[a] Main constituents of essential oils, determined with gas chromatography–mass spectometry.

[b] Numbers represent the *Eucalypus* species: 1, *E. badjensis;* 2, *E. badjensis x E. nitens;* 3, *E. botryoides;* 4, *E. darlympleana;* 5, *E. dorrigoensis;* 6, *E. elata;* 7, *E. fastigata;* 8, *E. fraxinoides;* 9, *E. nobilis;* 10, *E. oblicua;* 11, *E. polybractea;* 12, *E. radiata;* 13, *E. resinifera;* 14, *E. robertsoni;* 15, *E. rubida;* 16, *E. smithii.*

[c] Other minor compounds identified by matching the mass spectra with Wiley and NIST libraries were α-thujene, β-pinene, α-terpinene, linalool, spathulenol, and 10-epi-γ-eudesmol.

Regression analysis showed a significant relationship between the fumigant activity of the essential oils [$KT_{50(min)}$] and their corresponding concentration of 1,8-cineole (percentage), $KT_{50(min)} = 21.1 \pm 0.65 - 0.21 \pm 0.011 \times$ 1,8-cineole (percentage) ($r^2 = 0.84$; $F = 340.44$, df = 65, $P < 0.01$) (Fig. 2).

p-Cymene and 4-terpineol also showed a significant association, with the $KT_{50(min)}$ values ($r^2 = 0.59$; $F = 91.98$, df = 65, $P < 0.01$ and $r^2 = 0.16$; $F = 11.84$, df = 65, $P < 0.01$, respectively). α-Terpineol and α-pinene showed no statistically significant association between the concentration of the pure component and KT_{50} values.

To find the best fit statistical model, we performed a multiple regression analysis, but we observed that incorporating a variable not only increased the percentage of r^2 but also significantly decreased the *F* value. Thus the simple linear regression model with the independent variable 1,8-cineole (percentage) proved to be the best fit for the KT_{50} for horn flies.

These results show the importance of the 1,8-cineole component for the fumigant activity of *Eucalyptus* essential oils against *H. irritans.* As the fumigant activity of the oils increases, the oils become richer in 1,8-cineole.

Table 3. Fumigant activity of the essential oil vapors from different species of *Eucalyptus* against *H. irritans*

Essential oil	Fumigant activity			
	KT_{50} (CI) min[a]	Statistics[b]		
		Slope ± SE	χ^2	df
E. polybractea	3.44 (3.28–3.61)	8.53 ± 0.85	2.14	7
E. darlympleana	4.84 (4.59–5.09)	8.35 ± 0.81	2.07	6
E. smithii	5.70 (5.44–6.00)	6.11 ± 0.44	10.69	13
E. robertsonii	5.72 (5.38–6.03)	7.49 ± 0.84	1.78	7
E. badjensis × *E. nitens*	5.78 (5.47–6.10)	7.03 ± 0.64	4.96	7
E. radiata	6.20 (5.72–6.71)	6.30 ± 0.63	9.52	8
E. badjensis	6.36 (5.63–7.20)	6.23 ± 0.55	27.58	9
E. dorrigoensis	6.41 (6.15–6.67)	6.97 ± 0.54	6.28	9
E. rubida	6.54 (6.21–6.87)	9.38 ± 0.98	2.71	6
E. resinifera	7.16 (6.29–8.16)	4.11 ± 0.29	43.38	13
E. fastigata	14.48 (13.94–15.06)	5.75 ± 0.35	11.85	20
E. nobilis	14.91 (14.38–15.44)	4.89 ± 0.22	18.69	28
E. botryoides	16.19(15.72–16.67)	6.02 ± 0.29	9.15	21
E. fraxinoides	17.53 (16.88–18.22)	5.98 ± 0.33	22.87	20
E. obliqua	19.09 (17.95–20.40)	6.17 ± 0.36	68.08	24
E. elata	26.08 (25.16–27.13)	6.75 ± 0.35	42.42	27

[a] Time to 50% knockdown with a 95% CI. KT_{50} values are the means of four replicates by using 13–15 adults (total 54–60 adults) for each essential oil, determined at 1-min intervals until 100% of the adults were knocked down. Note: A lower knockdown time represents a higher toxicological activity.

[b] Statistics of the probit analysis of knockdown times.

Table 4. Fumigant activity of the individual oil components from different species of *Eucalyptus* against *Haematobia irritans* in comparison with dichlorvos

Oil component	Fumigant activity			
	KT_{50} (CI) min[a]	Statistics[b]		
		Slope ± SE	χ^2	df
1,8-Cineole	3.29 (2.75–3.86)	5.37 ± 0.49	23.14	6
p-Cymene	6.16 (5.84–6.50)	5.12 ± 0.38	3.70	10
α-Pinene	24.89 (24.18–25.62)	6.72 ± 0.36	26.37	28
Dichlorvos[c]	1.45 (1.33–1.56)	7.91 ± 1.03	2.12	3

[a] Time to 50% knockdown with a 95% CI. KT_{50} values are the means of four replicates by using 13–15 adults (total 54–60 adults) for each essential oil, determined at 1-min intervals until 100% of the adults were knocked down.

[b] Statistics of the probit analysis of knockdown times.

[c] Positive control.

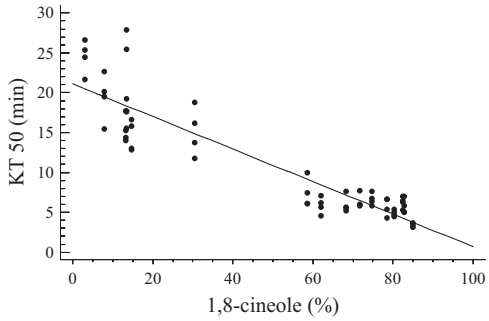

Fig. 2. Relationship between fumigant activity (KT_{50}) and the percentage of 1,8-cineole in essential oils from 16 species of *Eucalyptus*. Each point represents the KT_{50} value for each species of *Eucalyptus* and its corresponding percentage of 1,8-cineole.

The toxic effect of volatile substances penetrating through the insect spiracles as part of the respiratory process is strongly associated with their rate of volatility. Toloza et al. (2008) determined the main components of the essential oils extracted from *E. grandis*, *E. camaldulensis*, *E. tereticornis*, *E. grandis* × *E. tereticornis*, and *E. grandis* × *E. camaldulensis* and determined the fumigant activity on *Pediculus humanus capitis* De Geer. An increase in fumigant activity was explained by the presence of 1,8-cineole in the essential oils, but the addition of α-pinene as a variable had a stronger effect on the regression equation Toloza et al. (2006) studied the fumigant activity of 21 monoterpenes against *P. humanus capitis* and concluded that the most effective components were 1,8-cineole and anisole.

Many studies have reported similar results. Tarelli et al. (2009) studied the fumigant toxicity of five monoterpenes and five commercial essential oils against *Musca domestica* (L.) and concluded that the generic essential oil of *Eucalyptus*, rich in 1,8-cineole, was most effective. Sfara et al. (2009) evaluated the fumigant activity of five essential oils and seven monoterpenes on the first-instar nymphs of *Rhodnius prolixus* (Ståhl), and demonstrated that the generic essential oil of *Eucalyptus* spp. and the pure 1,8-cineole component was most effective. Rice and Coats (1994) established a polynomial correlation between the vapor pressure and the fumigant toxicity of monoterpenoids against *M. domestica.*

In conclusion, the results of this study show that 1,8-cineole and *Eucalyptus* essential oils with an elevated concentration of this monoterpene present a high knockdown activity against *H. irritans.* This botanical could be considered potentially useful for horn fly control in cattle. There is a possibility to develop new slow-release ear tag formulations containing 1,8-cineole as an active ingredient. The matrix should be formed by a combination of polymers and solvents that permit the gradually release of the active ingredient to place on the cattle as a spot-on treatment. Work is in progress to formulate the active components of *Eucalyptus* essential oils in long-lasting formulations adequate to provide residual treatments on beef and milk cattle.

Acknowledgments

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References Cited

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream, IL.

Barros, A.T.M., A. Gomes, and W. W. Koller. 2007. Insecticide Susceptibility of horn flies, *Haematobia irritans* (Diptera: Muscidae), in the state of Mato Grosso de Sul, Brazil. Bras. J. Vet. Parasitol. 16: 145–151.

Campbell, J. B., and G. D. Thomas. 1992. The history, biology, economics and control of the horn fly *Haematobia irritans* (L). Agric. Pract. 13: 31–36.

Coppen, J.J.W. 2002. Preface, pp. XI-XIV *In* J.J.W. Coppen (ed.), *Eucalyptus:* the genus *Eucalyptus.* Medicinal and aromatic plants—industrial profiles, vol. 22. Taylor & Francis, London and New York.

Denny, E.F.K. 2002. Distillation of *Eucalyptus* leaf oils, pp. 161–180. *In* J.J.W. Coppen (ed.), *Eucalyptus:* the genus *Eucalyptus.* Medicinal and aromatic plants—Industrial profiles, vol. 22. Taylor & Francis, London and New York.

Enam, E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. C 130: 325–337.

Franzios, G., M. Mirotson, E. Hatziapostolou, J. Kral, Z. G. Scouras, and T. P. Mavragani. 1997. Insecticidal and genotoxic activities of mint essential oils. J. Agric. Food Chem. 45: 2690–2694.

Guglielmone, A. A., E. Gimeno, J. Idiart, W. F. Fisher, M. M. Volpogni, O. Quaino, O. S. Anziani, S. G. Flores, and O. Warnke. 1999. Skin lesions and cattle hide damage from *Haematobia irritans* infestations in cattle. Med. Vet. Entomol. 13: 323–328.

Guglielmone, A. A., M. E. Castelli, M. M. Volpogni, A. J. Mangold, P. D. Medus, and O. S. Anziani. 2001a. Comparación de la concentración letal 50 de diazinón y cipermetrina para *Haematobia irritans* (Díptera: Muscidae) entre áreas de producción de leche o carne de Santa Fé y Entre Ríos, Argentina. Rev. Med. Vet. (Buenos Aires) 82: 209–211.

Guglielmone, A. A., M. E. Castelli, M. M. Volpogni, A. J. Mangold, O. S. Anziani, J. R. Martis, P. D. Medus, and V. H. Suárez. 2001b. Toxicity of cypermethrin and diazinon to *Haematobia irritans* (Diptera: Muscidae) in its American southern range. Vet. Parasitol. 101: 67–73.

Heine, J. 1987. Flies as parasites on grazing cattle in Europe. Vet. Med. Rev. 1: 9–16.

Hillerton, J. E. 1988. Distribution of cattle-visiting Muscidae (Diptera) in Great Britain. Entomologist 1: 57–67.

Isman, M. B. 1999. Pesticides based on plant essential oils. Pestic. Outlook 10: 68–72.

Isman, M. 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45–66.

Jensen, K.M.V., J. B. Jespersen, M. A. Birkett, J. A. Pikett, G. Thomas, L. J. Wadhams, and C. M. Woodcock. 2004.

Variation in the load of the horn fly, Haematobia irritans, in cattle herds is determined by the presence or absence of individual heifers. Med. Vet. Entomol. 18: 275–280.

Kunz, S. E., J. A. Miller, P. L. Sims, and D. C. Meyerhoffer. 1984. Economics of controlling horn flies (Diptera: Muscidae) in range cattle management. J. Econ. Entomol. 77: 657–660.

Kuramochi, K. 2000. Ovipositional behavior of the horn fly (Diptera: Muscidae) in the field. J. Med. Entomol. 37: 461–466.

Litchfield, J. T., and F. A. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99–113.

Lucia, A., S. Licastro, E. Zerba, P. Gonzalez Audino, and H. Masuh. 2009. Sensitivity of *Aedes aegypti* adults (Diptera: Culicidae) to vapors of *Eucalyptus* essential oils. Bioresour. Technol. 100: 6083–6087.

Lucia, A., S. Licastro, E. Zerba, and H. Masuh. 2008. Yield, chemical composition and bioactivity of essential oils from twelve species of *Eucalyptus* on *Aedes aegypti* (L.) larvae (Diptera: Culicidae). Entomol. Exp. Appl. 129: 107–114.

Luzuriaga, R., C. Eddi, J. Caracostantogolo, E. Botto, and J. Pereira. 1991. Diagnóstico de parasitación con *Haematobia irritans* (L.) en bovinos de Misiones, República Argentina. Rev. Med. Vet. (Buenos Aires) 72: 262–263.

McKenzie, C. L., and R. L. Byford. 1993. Continuous, alternating, and mixed insecticides affect development of resistance in the horn fly (Diptera: Muscidae). J. Econ. Entomol. 86: 1040–1048.

Mill, P. J. 1985. Structure and physiology of the respiratory system, pp. 517–593. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 3. Pergamon, Oxford, United Kingdom.

Picollo, M. I., A. C. Toloza, G. Mougabure Cueto, J. Zygadlo, and E. Zerba. 2008. Anticholinesterase and pediculicidal activities of monoterpenoids. Fitoterapia 79: 271–278.

Rice, P. J., and J. R. Coats. 1994. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87: 1172–1179.

Riley, C. W. 1889. The horn fly *Haematobia serrata* (Robineau Desvoidy). Rev. Insect Life 2: 93–103.

Schreiber, E. T., J. B. Campbell, S. E. Kunz, D. C. Clanton, and D. B. Hudson. 1987. Effects of horn fly (Diptera: Muscidae) control on cows and gastrointestinal worm (Nematode: Trichostrongylidae) treatment for calves on cow and calf weight gains. J. Econ. Entomol. 80: 451–454.

Sfara, V., E. N. Zerba, and R. A. Alzogaray. 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus.* J. Med. Entomol. 46: 511–515.

Singh, G., and R. K. Upadhyay. 1993. Essential oils: a potent source of natural pesticides. J. Sci. Indian Res. 25: 676–283.

Tarelli, G., E. N. Zerba, and R. A. Alzogaray. 2009. Toxicity to vapor exposure and topical application of essential oils and monoterpenes on *Musca domestica* (Diptera: Muscidae). J. Econ. Entomol. 102: 1383–1388.

Thomsen, M. 1938. Stuefluen (*Musca domestica*) og stickfluen (*Stomoxys calcitrans*). 176. Beretning fra Eorsögslaboratoriet. Kopenhagen, Bangs Forlag. 352 S., 107 Textfig., 13 Taf.

Toloza, A. C., J. Zygadlo, G. Mougabure Cueto, F. Biurrun, E. Zerba, and M. I. Picollo. 2006. Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. J. Med. Entomol. 43: 889–895.

Toloza, A. C., A. Lucia, E. Zerba, H. Masuh, and M. I. Picollo. 2008. Interspecific hybridization of *Eucalyptus* as a potential tool to improve the bioactivity of essential oils against permethrin-resistant head lice from Argentina. Bioresourc. Technol. 99: 7341–7347.

Vogelsang, E. G., and J. C. De Armas. 1940. La mosquilla del ganado, *Lyperosia irritans* (L.) en Venezuela. Rev. Med. Vet. Parasitol. 2: 95–98.

Weinzieri, R. A. 2000. Botanical insecticides, soaps and oils. *In* J. E. Rechcigl and N. A. Rechcigl (eds.), Biological and biotechnological control of insect pest. Lewis Publishers. Boca Raton, FL.

Weinzieri, R. A., D. Palevitch, and L. Craker. 1994. Volatile oils as potential insecticides. Herbs, Spice Med. Plant Dig. 12: 1–8.

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