Changes in Chemical Composition of Essential Oils from Leaves of Different *Lantana camara* L. (Verbenaceae) Varieties after Feeding by the Introduced Biological Control Agent, *Falconia intermedia* Distant (Hemiptera: Miridae)

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Changes in chemical composition of essential oils from leaves of different *Lantana camara* L. (Verbenaceae) varieties after feeding by the introduced biological control agent, *Falconia intermedia* Distant (Hemiptera: Miridae)

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Lantana camara L. (Verbenaceae) is one of the most problematic plant invaders in South Africa and has been targeted for biological control for over 50 years. Essential oil constituents which often change in response to insect herbivory are reported to play a crucial role in plant–insect interactions. However, nothing is known about the chemical profiles of essential oils of *L. camara* varieties in South Africa and how this changes under herbivory. Therefore, essential oils were collected using hydrodistillation from undamaged and insect-damaged leaves of four *L. camara* varieties and analysed using gas chromatography-mass spectrometry to elucidate their chemical profiles. A total of 163 compounds were identified from the undamaged leaves of the various *L. camara* varieties. Feeding by the biocontrol agent *Falconia intermedia* Distant (Hemiptera: Miridae) resulted in changes in the quality and quantity of chemical constituents of the essential oils. Only 75 compounds were identified from the insect-damaged leaves of *L. camara* varieties. Terpenes were the major components across the varieties, while caryophyllene, hexane, naphthalene, copaene and α-caryophyllene were common in all the varieties tested from both undamaged and insect-damaged leaves. Results from this study indicated the chemical distinctiveness of the Whitney Farm variety from other varieties. The changes in chemical concentrations indicated that feeding by the mirid on *L. camara* varieties causes an induction by either reducing or increasing the chemical concentrations. These inductions following the feeding by *F. intermedia* could be having a negative impact on the success of biological control against *L. camara* varieties. However, the focus of this paper is to report on the chemical baseline of *L. camara* varieties. Hence, comparisons of chemical compound concentrations of *L. camara* essential oils tested and the feeding-induced changes with respect to their quality and quantity are discussed.

Key words: Miridae, South Africa, hydrodistillation, GC-MS, Verbenaceae.

INTRODUCTION

Lantana camara L. (Verbenaceae), also known as lantana or wild sage, originates from South and Central America (Holm *et al*. 1977; Stirton 1977; Ghisalberti 2000; Day *et al*. 2003a). It is widely considered to be one of the worst weeds in the world (Holm *et al*. 1977). Lantana invades forest edges, sand dunes, beach fronts and many other habitats or conditions, where it forms impenetrable thickets (Cilliers 1983; Henderson 1995; Kohli *et al*. 2006; Prasad 2012). It degrades the natural biodiversity and reduces the value of agricultural land (Wells & Stirton 1988; Henderson 1995; Day *et al*. 2003b) and has consequently been a target for biological control for over 50 years (Urban *et al*. 2011). The success of this has been variable, with several factors decreasing effectiveness. These include climatic incompatibility between the country of origin and new range, variable susceptibility of the different plant genotypes, parasitism and predation and inappropriate release strategies (Sands & Harley 1980; Neser & Cilliers 1989; Day & Neser 2000; Urban & Phenye 2005; Tourle 2010).

The sap-sucking bug, *Falconia intermedia* Distant (Hemiptera: Miridae) was released on five different varieties of the weed, each at a separate site, in the Eastern Cape Province of South Africa in 2001 to improve the level of biocontrol (Heshula & Hill 2011). The variety of *L. camara* at each of these sites was named according to the site's locality, namely the East London variety, the Whitney Farm variety, the Lyndhurst variety, the Port Alfred variety and the Heather Glen variety. *Falconia intermedia* populations established on three varieties: East London, Whitney Farm

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(Heshula 2005) and Port Alfred (Heshula, pers. obs.). However, this agent was less successful than anticipated and this was ascribed to induced plant defences (Heshula & Hill 2011, 2014). Following feeding by *F. intermedia*, increases in physical plant defences, namely leaf toughness and trichome density, both of which deter and hinder herbivores from feeding and attaching to or moving on the plant were reported (Heshula & Hill 2011). Not only did *L. camara* display induced physical responses, but the preliminary results reported that there were also induced chemical responses displayed by some lantana varieties (Heshula & Hill 2014).

Chemical compounds in plant essential oils are reported to play a crucial role in plant ecology (Hyldgaard *et al*. 2012; Rajendran *et al*. 2014; Tisserand 2015). Plants use chemical compounds as secondary metabolites for protection against fungi, bacteria and herbivorous insects and, for communicating amongst each other (Tajkarimi *et al*. 2010; Rajendran *et al*. 2014; Tisserand 2015). Many studies have been conducted on the chemical profiles of *L. camara* around the world (Seth *et al*. 2012; Sousa *et al*. 2012) but not in South Africa where different varieties of the weed are present. Therefore, the aim of this study was to determine if feeding by the biocontrol agent, *F. intermedia*, caused differences in the chemical profile of *L. camara* leaves that would suggest induced chemical defences.

MATERIAL AND METHODS

Plant material

Falconia intermedia was released at five sites in the Eastern Cape Province of South Africa to control *L. camara*, including three coastal sites: East London (32°58′20″S 27°57′24″E), Port Alfred (33°36′16″S 26°52′16″E) and Whitney Farm (33°40′43″S 26°35′49″E); and two inland sites: Heather Glen Farm (33°19′28″S 26°47′31″E) and Lyndhurst Farm (33°27′11″S 26°53′10″E) (Heshula 2005, 2009). Forty lantana cuttings were taken from the plants at each of these sites. At each of these sites, only one variety of lantana was present. The tip of each cutting was dipped into water then into root growth hormone (Dip and Grow®, Fleuron (Pty) Ltd, South Africa). The cuttings were planted in a plant pot of about 200 mm in diameter filled with planting soil and transported to the greenhouse in Waainek Research Laboratory at Rhodes University. Cuttings were watered daily and fertilised fortnightly using Seagro® until leaves started to sprout and roots to grow, after which they were transplanted into individual pots. Plants were continuously watered daily and fertilised fortnightly. The plants were grown into mature plants with a size range of 50–70 cm. From 40 cuttings collected from each site and grown into mature plants, 16 plants were caged with *F. intermedia* for insect rearing. The remaining 24 mature plants from each variety were used for the trials. In each trial, four plants from each variety were used as controls (insect-free) and four as treatments (damaged leaves). The plants were kept free of insect feeding by spraying them with insecticide (malathion DP.).

Insects

A colony of *F. intermedia* was collected from the East London site where the population had persisted for over a decade. Branches with *F. intermedia* were removed and transported to the laboratory in pillowcases. The insects were reared in cages each containing four healthy plants representing each variety in the greenhouse of the Waainek Research Laboratory, Rhodes University. For experiments, three to four adult mirids per leaf were released into the cages to feed continuously for 21 days. After 21 days, the mirids were removed from the damaged plants and placed on new healthy plants to maintain the culture. Samples of 450–500 g of leaves were then removed from the treatment (damaged leaves) and control (insect-free) plants to run the extractions.

Essential oils extraction

Essential oils were extracted from leaves of the five *L. camara* varieties, in which 450–500 g of fresh plant leaves were immersed in distilled water in a round-bottom flask (Seth *et al*. 2012; Ranjitha & Vikiyalakshimi 2014). The contents of the round-bottom flask were heated to 70 °C by means of a heating mantle. In the condensation process, the water and oil separate (Saikia & Sohoo 2011; Sousa *et al*. 2012) allowing the oil to be collected. Essential oils were thus extracted from treatment (damaged leaves) and control (insect-free) plants of all five varieties. After 5 h of heating had elapsed, an extra 1½ h was allowed for cooling down. Afterwards, essential oils were collected from the hydrosol. Excess water was then dried up by anhydrous sodium sulphate (Na_2SO_4) and the oils were stored in refrigerator at 4 °C until analysis. The essential oil yields of the Lyndhurst variety and damaged leaves of the Heather Glen variety were not enough to be detected by the GC-MS; hence, there are no results to show for these treatments.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were obtained using a Hewlett Packard 5973 MSD equipped with a capillary column (30 m, 0.25 mm i.d., 0.25 μm). The initial temperature was 45 °C which was then increased to 240 °C at the rate of 15 °C/min. The carrier gas used was helium at the flow rate of 1 ml/min. The chemical compounds in the essential oil samples were identified by comparing identical retention times and chromatogram peaks using the NIST10/HPPEST/WILEY 275 mass spectral libraries. Chemical compounds were identified from the essential oils of insect-free leaves and insect-damaged leaves to compare the variations in their concentrations and their retention times.

RESULTS

Chemical compounds in undamaged leaves of lantana varieties

The essential oils were obtained by means of hydrodistillation from the undamaged and damaged leaves of the different *L. camara* varieties. One-hundred and sixty-three chemical compounds were identified from undamaged leaves compared to 75 chemical compounds from damaged leaves (Table 1). There were different classes of compounds identified from undamaged leaves across the four varieties tested. Terpenes were the dominant compounds, notably sesquiterpenes followed by monoterpenes. The Whitney Farm variety had the highest number of compounds (56), followed by the East London variety (41), Heather Glen variety (36) and Port Alfred variety (30). Not only did the Whitney Farm variety display the highest number of compounds, it also contained the highest number of unique compounds (22) when compared with the other varieties. Thus, the Whitney Farm variety was chemically distinct from the other varieties tested. The chemical distinctiveness of the variety can be used to distinguish between different lantana varieties. Furthermore, it suggests that different chemical responses against herbivores could be expected from plants of this variety that would not be seen in the other three.

The two dominant compounds in the essential oils of each lantana variety were used to characterise each variety, as follows. The East London variety was characterised by hexane and methyl cyclopentane with concentrations of 71.20 % and 9.00 %, respectively. The Port Alfred variety was characterised by 1,6-cyclodecadiene and caryophyllene with the highest concentration of 18.20 % and 15.70 %, respectively. The Whitney Farm variety was characterised by caryophyllene and α-muurolene with the highest concentration of 15.10 % and 7.10 %, respectively. The Heather Glen variety was characterised by caryophyllene and 5-hepten-3-one,2-(5-ethenyltetrahydro-5-methyl -2-furanyl)-6-methyl-,2S- [2.alpha.(R*),5.alpha]] with the highest concentration of 15.60 % and 20.40 %, respectively.

Comparisons of chemical concentrations in undamaged and damaged leaves of lantana varieties

Notable changes in the identities and numbers of essential oil compounds and in their concentrations were measured across the three lantana varieties following feeding by the mirid, *F. intermedia*. Some compounds were only identified prior to feeding and other compounds only post-feeding, while other chemicals were present in both leaf states (Table 1). The East London variety contained 41 compounds in undamaged leaves and 39 compounds in damaged leaves. The concentrations of chemical compounds found before and after feeding, were reduced in hexane from 71.20 % to 33.30 %, methyl cyclopentane from 8.62 % to 3.80 %, 3-hexen-1-ol,(Z) from 4.40 % to 3.20 % and 1,6,10-dodecatrien-3-ol,3,7,11,trimethyl-[S-(Z)] from 5.44 % to 0.40 %. Increases in chemical concentrations were seen in compounds such as copaene from 0.64 % to 2.72 %, caryophyllene from 3.13 % to 7.60 %, 5-hepten-3-one, 2-(5-ethenyltetrahydro-5-ethenyltetra-hydro-5-methyl from 1.11 % to 11.30 %, naphthalene from 0.21 % to 1.62 % and cycloisolongifolene from 0.17 % to 1.40 %.

The Port Alfred variety contained 30 compounds in undamaged leaves *versus* 19 compounds in damaged leaves. Reductions in chemical concentrations were seen in naphthalene from 4.80 % to 0.92 %, caryophyllene from 15.70 % to 4.30 %, di-epi-α-cedrene from 9.61 % to 1.50 % and α-caryophyllene from 9.30 % to 1.90 %. In contrast, concentrations of several other chemicals increased

Table 1. The chemical compounds in the essential oils of leaves of undamaged (Und) and damaged (Dam) Lantana camara varieties. East London (EL); Port Alfred (PA); Whitney Farm (WF); Heather Glen (HG); retention time in minutes (Rt/min); concentration of compounds in essential oils (%).

Compound	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%
Pentane, 2-methyl	–	–	1.69	0.23	–	–	–	–	1.694	0.06	–	–	–	–
Pentane, 3-methyl	–	–	1.755	2.00	1.736	0.15	1.736	2.56	1.759	0.50	1.739	2.07	–	–
Hexane	1.811	71.19	1.830	33.30	1.811	6.13	1.805	75.81	1.839	6.95	1.814	51.93	–	–
Methyl cyclopentane	1.993	8.62	2.017	3.77	2.003	0.32	1.998	5.47	2.021	0.92	2.006	4.19	–	–
Cyclohexane	2.271	0.34	2.295	0.14	–	–	–	–	–	–	–	–	–	–
2-Hexenal, (E)-	–	–	–	–	–	–	–	–	6.342	0.03	–	–	–	–
3-Hexen-1-ol, (Z)-	6.400	4.38	6.419	3.17	–	–	–	–	6.417	0.28	–	–	–	–
1-Hexanol	–	–	–	–	–	–	–	–	6.760	0.03	–	–	–	–
Ethanol, 2-butoxy	–	–	–	–	–	–	–	–	7.503	0.55	–	–	–	–
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-	–	–	–	–	–	–	–	–	7.888	0.04	–	–	8.290	4.26
1R-α-Pinene	7.472	0.13	8.023	0.30	–	–	–	–	8.027	0.45	–	–	7.477	1.10
Camphene	7.819	0.07	8.328	0.17	–	–	–	–	8.332	0.16	–	–	7.819	0.57
Bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl)	–	–	8.729	2.53	–	–	–	–	8.985	0.11	–	–		–
β-Pinene	8.365	0.11	8.810	0.28	–	–	–	–	8.813	0.34	–	–	8.370	0.82
1-Octen-3-ol	8.871	0.16	–	–	–	–	–	–	8.872	0.08	–	–	8.427	0.28
Bicyclo[4.1.0] hept-3-ene, 3,7,7-trimethyl	–	–	9.291	0.59	13.070	1.59	–	–	9.295	0.42	–	–	–	–
Benzene, 1-methyl-3-(1-methylethyl)	–	–	9.537	0.09	–	–	–	–	9.541	0.05	–	–	9.194	0.27
β-Myrcene	8.590	0.06	–	–	–	–	–	–	–	–	–	–	8.590	0.33
3-Carene	8.911	0.20	10.537	0.21	–	–	–	–	9.295	0.42	–	–	8.916	1.40
β-Phellandrene	–	–	8.986	0.17	–	–	–	–	–	–	–	–	–	–
D-Limonene	9.258	0.11	–	–	–	–	–	–	–	–	–	–	9.264	0.67
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl	–	–	–	–	–	–	–	–	9.418	0.03	–	–	–	–
Eucalyptol	9.647	0.39	9.655	1.84	–	–	–	–	9.658	1.12	–	–	9.328	1.22
1,3,6-Octatriene, 3,7-dimethyl-, (Z)	9.921	0.08	9.820	0.19	–	–	–	–	9.819	0.32	–	–	9.526	0.35

Continued on p. 466

Table 1 (continued)

Compound	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	9.708	0.05	10.190	0.24	–	–	–	–	10.001	0.08	–	–	9.713	0.15
1,6-Octadien-3-ol, 3,7-dimethyl	10.312	0.10	–	–	–	–	–	–	–	–	–	–	10.312	0.15
Cyclohexene, 1-methyl-4-(methylethylidene)	–	–	–	–	–	–	–	–	10.359	0.08	–	–	–	–
Bicyclo[2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)	11.166	0.49	11.168	0.96	–	–	–	–	11.17	0.41	–	–	10.996	0.72
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	11.530	0.18	11.532	0.24	–	–	–	–	–	–	–	–	11.392	0.40
1,3,7-Octatriene, 3,7-dimethyl	9.520	0.07	–	–	–	–	10.541	0.55	–	–	–	–	–	–
3-Cyclohexene-1-methanol, .alpha. alpha. 4-trimethyl-	11.569	0.09	11.687	0.25	–	–	11.691	0.05	–	–	–	–	–	–
Bicyclo[3.1.1] hept-3-en-2-one, 4,6,6-trimethyl-, (1S)	11.840	0.11	11.837	0.16	–	–	11.333	0.02	–	–	–	–	–	–
(+)-4-Carene	–	–	–	–	–	–	10.359	0.08	–	–	–	–	–	–
Borneol	11.307	0.11	–	–	–	–	11.461	0.13	–	–	–	–	–	–
6-Octen-1-ol,3,7-dimethyl	–	–	–	–	–	–	11.946	0.74	–	–	–	–	–	–
Bornyl acetate	–	–	–	–	–	–	–	–	12.590	0.02	–	–	–	–
1,5,5-Trimethyl-6-methylene-cyclohexene	–	–	–	–	–	–	–	–	12..959	0.04	–	–	–	–
Phenol,2-methoxy-3-(2-propenyl)	–	–	–	–	13.251	011	–	–	–	–	–	–	–	–
α-Muurolene	–	–	–	–	–	–	–	–	14.617	7.50	–	–	–	–
Copaene	13.532	0.64	14.736	2.72	13.481	1.57	13.499	1.20	13.499	1.20	13.479	0.70	13.532	0.57
α-Cubebene	13.193	0.12	13.490	0.44	13.182	0.34	–	–	13.194	0.19	15.790	0.54	13.532	0.57
1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1a.α.,7.α.,7a.α.,7b.α)]	–	–	–	–	13.585	3.18	13.578	0.57	13.606	2.64	13.581	1.19	13.644	1.32
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*, S*)]-	–	–	–	–	13.663	0.83	–	–	–	–	–	–	13.730	0.40

Continued on p. 467

Table 1 (continued)

Compound	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%
1H-Benzocycloheptene, 1,2,4a,5,6,7,8-hexahydro	–	–	–	–	13.781	0.19	–	–	–	–	–	–	–	–
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	–	–	–	–	–	–	–	–	–	–	–	–	13.842	0.54
1H-Cyclopental[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.α.,3b.β.,4.β.,7.α.,7aS	–	–	–	–	–	–	13.984	0.49	–	–	–	–	14.083	1.70
γ-Himachalene	–	–	–	–	–	–	–	–	13.798	0.32	–	–	–	–
Tricyclo[5.4.0.0 (2,8)] undec-9-ene, 2,6,6,9-tetramethyl	–	–	13.784	0.15	–	–	–	–	–	–	–	–	14.880	3.47
Caryophyllene	13.926	3.13	13.923	7.59	13.931	15.70	13.909	4.02	13.996	15.06	13.912	11.10	14.013	15.56
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	14.001	0.29	14.003	0.90	–	–	–	–	14.499	7.32	13.987	1.44	–	–
Cyclohexene, 3-(1,5-dimethyl-4- -hexenyl)-6-methylene-,[S-(R*,S*)]	–	–	–	–	14.070	1.21	–	–	–	–	–	–	–	–
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)	–	–	14.078	0.40	–	–	–	–	–	–	–	–	14.174	0.41
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14.375	0.83	14.372	4.52	–	–	–	–	–	–	–	–	14.500	8.00
(+)-Epi-bicyclosesquiphellandrene	–	–	–	–	13.995	3.37	–	–	–	–	–	–	–	–
α-Caryophyllene	14.345	1.54	14.244	0.78	14.241	9.03	14.225	1.89	14.290	7.01	14.228	5.48	14.350	6.39
1H-3a, 7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8,tetramethyl-,[3R-(3.α.,3a.β.,7.β.,8a.α.)]	–	–	14.607	3.25	16.102	0.61	14.337	2.44	14.397	5.11	14.597	1.68	–	–
5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl-, [2S-[2.alpha.(R*),5.alpha.]]	15.177	1.11	15.185	1.03	–	–	–	–	–	–	–	–	15.382	20.44
Epizonarene	–	–	15.260	0.54	–	–	14.064	0.51	–	–	14.554	1.88	–	–
Aromadendrene	14.338	0.34	–	–	–	–	–	–	–	–	–	–	–	–

Continued on p. 468

Table 1 (continued)

Compound	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%
Di-epi-α-cedrene (I)	–	–	–	–	14.359	11.11	–	–	–	–	14.335	5.67	–	–
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-[S-(E,E)]-	14.455	0.57	–	–	14.460	18.22	–	–	14.499	7.32	14.442	7.45	14.575	2.87
Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-(S)-	–	–	–	–	15.054	0.40	–	–	14.617	7.50	–	–	–	–
1,5-Heptadiene, 2,5-dimethyl-3-methylene	–	–	–	–	–	–	–	–	–	–	–	–	14.708	6.91
1,3-Cyclohexadiene, 5-(1,5-dimethyl)	–	–	–	–	13.663	0.83	14.471	2.28	13.071	0.83	–	–	14.612	2.76
1,4-Methanoazulene, decahydro-4,8,8-trimethylene	–	–	–	–	14.974	0.80	–	–	–	–	–	–	–	–
10s, 11s-Himachala-3(12), 4-diene	–	–	–	–	–	–	–	–	15.002	0.94	–	–	–	–
Caryophyllene oxide	16.757	0.89	15.330	1.09	15.316	0.76	–	–	–	–	15.314	0.37	15.532	2.17
Nerolidol/1,6,10-Dodecatrien-3-ol,3,7,11,trimethyl-,[S-(Z)]-	15.217	5.44	14.078	0.40	20.895	1.74	–	–	14.109	1.01	–	–	15.184	3.05
1H-Cycloprop[e]azulene, decahydro, 1,7-decahydro-1,trimethyl-4-methylene-, [1aR-(1a.α.,4aβ.7.α.,7aβ.,7b.α)]	15.429	0.16	–	–	15.947	0.47	–	–	15.440	0.95	–	–	–	–
Isoledene	14.568	1.09	15.260	0.54	14.549	0.62	–	–	14.178	0.45	14.554	1.88		–
β-Humulene	–	–	–	–	15.412	0.33	–	–	–	–	–	–	–	–
γ-Elemene	15.308	1.08	15.118	1.74	15.118	1.74	–	–	–	–	15.111	0.54	–	–
Di-epi- α-cedrene	–	–	–	–	14.605	9.61	14.594	1.47	–	–	–	–	–	–
β-Guaiene	–	–	15.608	1.53	–	–	–	–	–	–	–	–	–	–
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R(1.alpha.,3aβ.,4.α.,7.β)]	15.671	0.87	–	–	–	–	–	–	15.077	0.64	–	–	15.644	0.62
(Z,Z)-α-Fernesene	15.719	1.10	–	–	–	–	–	–	–	–	–	–	–	–
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl	–	–	–	–	–	–	–	–	–	–	–	–	15.789	0.67

Continued on p. 469

Table 1 (continued)

Compound	EL Und		EL Dam		PA Und		PA Dam		WF Und		WF Dam		HG Und	
	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%	Rt/min	%
α-Cardinol	–	–	–	–	–	–	–	–	–	–	–	–	16.174	0.29
Naphthalene	14.254	0.21	15.790	1.62	14.851	4.79	14.706	0.92	14.873	0.87	14.715	2.11	15.901	0.72
Isoaromadendrene epoxide	–	–	–	–	–	–	–	–	15.349	2.23	–	–	–	–
Guaia-3, 9-diene	–	–	–	–	–	–	–	–	15.628	1.46	–	–	–	–
Cycloisolongifolene, 8,9-dehydro	15.268	0.17	15.923	1.37	–	–	–	–	15.943	3.42	–	–	–	–
β-Panasinsene	15.429	0.16	–	–	–	–	–	–	–	–	–	–	–	–
2-Pentadecanone,6,10,14-trimethyl	–	–	–	–	–	–	–	–	17.724	0.05	–	–	–	–
9,12,15-Octadecatrienoic acid	–	–	–	–	–	–	–	–	18.527	0.08	–	–	–	–
1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl	–	–	–	–	20.895	0.11	–	–	14.109	1.01	–	–	–	–
n-Hexadecanoic acid	–	–	–	–	–	–	–	–	19.671	0.07	–	–	–	–
Phytol	22.737	1.60	–	–	22.210	0.82	–	–	–	–	–	–	–	–
1,6,10,14-Hexadecatetrene-3-ol,3,7,11,15-tetramethyl	–	–	–	–	–	–	–	–	20.939	0.25	–	–	–	–
Hexadecane	–	–	–	–	–	–	–	–	22.089	0.04	–	–	–	–
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	–	–	–	–	–	–	–	–	24.191	0.02	–	–	–	–
Octacosane	–	–	–	–	–	–	–	–	24.528	0.02	–	–	–	–

after herbivore damage, notably pentane, 3-methyl from 0.20 % to 2.60 %, hexane from 6.13 % to 76.00 %, methyl cyclopentane from 0.32 % to 5.50 %, 1H-cyclopropa[a]naphthalene from 0.44 % to 0.60 % and 1,3-cyclohexadiene,5-(1,5-dimethyl) from 0.83 % to 2.30 %.

The Whitney Farm variety had the highest number of compounds (56) in undamaged leaves but only 17 compounds in damaged leaves. Post-feeding reductions in concentrations of chemicals were recorded in copaene from 1.20 % to 1.00 %, 1H-cyclopropa[a]naphthalene from 2.64 % to 1.20 %, caryophyllene from 15.10 % to 11.10 %, bicyclo[4.4.0]dec-1-ene from 7.32 % to 1.44 %, and α-caryophyllene from 7.10 % to 5.50 %. Increases in chemical concentrations were recorded in pentane,3-methyl from 0.50 % to 2.10 %, hexane from 7.00 % to 52 %, methyl cyclopentane from 1.00 % to 4.20 %, 1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-[S(E,E)] from 7.32 % to 7.50 %, isoledene from 0.50 % to 2.00 %, naphthalene from 1.00 % to 2.1 1%, and α-cubebene from 0.20 % to 0.54 %.

The Heather Glen variety had 36 compounds identified from undamaged leaves. The essential oil chemicals comprised: copaene (1.00 %), α-cubebene (1.00 %), 1H-cyclopropa[a]naphthalene (1.32 %), caryophyllene (16.00 %), α-caryophyllene (6.40 %), 5-hepten-3-one,2-(5-ethenyltetrahydro-5-ethenyltetrahydro-5-methyl (20.44 %), 1,6-cyclodecadiene (2.87 %), 1,3-cyclohexadiene,5-(1,5-dimethyl) (3.00 %), caryophyllene oxide (2.20 %), 1,6,10-dodecatrien-3-ol,3,7,11,trimethyl-,[S(Z)] (3.10 %) and naphthalene (1.00 %).

DISCUSSION

Chemical compounds in the essential oils of four South African lantana varieties showed variations in both their numbers and their relative concentrations for both undamaged and damaged leaves. These findings were comparable with previous studies on various plant species in relation to the number of compounds and classes of chemicals identified (Mollenbeck *et al.* 1997; Ngassoum *et al.* 1999; Conti *et al.* 2008; Damasceno *et al.* 2010). Moreover, there was a consistent trend across the lantana varieties tested of a reduction in the total number of compounds following *F. intermedia* feeding. Similar reductions in chemical compounds after insect feeding were reported in *Brassica oleracea* var. *sabauda* (Brassicaceae) (Conti *et al.* 2008), *Baccharis spicata* (Asteraceae) and *Schinus polygamus* (Anacardiaceae) (Damasceno *et al.* 2010). However, such chemical responses to herbivory have never been reported for plants in the Verbenaceae, including *L. camara*. The total numbers of constitutive compounds expressed were higher than those implicated in induced defences, since the latter are costly as they require more energy for enzymatic processes than constitutive defences (Karban & Myers 1989; Baldwin 1998; Opitz *et al.* 2008). This may explain why there were more compounds in the constitutive rather than the feeding-induced state of the varieties tested. However, some compounds (hexane, copaene, caryophyllene, naphthalene and α-caryophyllene) were present in relatively high concentrations in both constitutive and induced states of the plants in each of the varieties. Caryophyllene has been identified in the chemical profiles of *L. camara* varieties from many studies (Khan *et al.* 2002; Montanari *et al.* 2011; Passos *et al.* 2012; Seth *et al.* 2012; Sousa *et al.* 2012; Zoubiri & Baalioumer 2012). In addition, research on different plant species has identified caryophyllene as one of the major plant compounds: in five *Bursera* species (Burseraceae) (Noge & Becerra 2009), in infested citrus fruit (Rutaceae) (Kendra *et al.* 2011; Van der Walt 2012) and in essential oils of *Commiphora leptophloeos* (Mart) J.B. Gillet (Burseraceae) (Da Silva *et al.* 2015). This is the first study to identify napthalene as one of the compounds present in the essential oils of lantana varieties. However, studies on *Juniperus* spp. (Cupressaceae) and *Eriotheca longitubulosa* A. Robyns (Bombacaceae) also identified naphthalene as one of the chemical components of their essential oils (Adams 1998; MacFarlane *et al.* 2003). Naphthalene is commercially used in insecticides and insect repellents (Bolton & Eaton 1968; Chen *et al.* 1998), and it thus likely that *L. camara* utilises this compound in a similar manner.

It is evident that the *L. camara* varieties tested display substantial variation in the chemical compounds contained in their essential oils and that these can be used to differentiate these varieties. Based on the results of this study, differences in the chemical ecology of the different lantana varieties could be negatively affecting the behaviour of *F. intermedia*. Such instances have been reported whereby plants under attack by herbivores emit chemical compounds that are used chiefly to deter these herbivores from further feeding by repellence, as well as to attract their natural enemies (De Moraes *et al*. 2001; Kessler & Baldwin 2001; Holopainen & Blande 2012; Dudareva *et al*. 2013; Kumari *et al*. 2014). Therefore, it is crucial to consider the influence of these newly identified chemical compounds on the behaviour of *F. intermedia* and this will be the focus of future research. In conclusion, studies on the chemical profiles of invasive plant species and their response to insect herbivory could be beneficial to the biological control programme. They could be used to understand the influence they might have on the biological control agents not to be successful to their control when such cases take place.

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