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Chemical profile of the volatiles of *Baccharis salicifolia* (Asteraceae) and interaction with *Macrodactylus nigripes* (Coleoptera:Melolonthidae)

Perfil químico de volátiles de *Baccharis salicifolia* (Asteraceae) e interacción con *Macrodactylus nigripes* (Coleoptera:Melolonthidae)

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Abstract

Plant products or substances mediate interactions among organisms from different trophic levels, including phytophagous insects. These interactions have been cited in a chemical-ecology context for species of Coleoptera Melolonthidae. However, there are no previous reports of these interactions among melolonthid beetles species distributed in Mexico and host plants. For the above, the interaction between adults of ‘rose chafer’ *Macrodactylus nigripes* Bates (Coleoptera:Melolonthidae) and ‘seep willow’ shrub *Baccharis salicifolia* (Ruíz and Pav.) Pers. (Asteraceae) was recorded and the leaves volatiles of seep willow that might be involved in the attraction of these insects were extracted and identified. The sequence of behavior patterns that conforms this interaction was described. Females of *M. nigripes* emerge from the soil and flight to the leaves of seep willow shrubs. Each female settled on leaf and they place their mandibles on the leaf margin and start moving them from right to left to obtain small leaf fragments to ingest. In addition, females exhibit a similar ‘calling’ behavior as well as the others species. Following the females, males emerge from the soil and repeat the females’ behavior, approaching them for mating. A list of the leaves volatiles of seep willow was generated by solid phase microextraction (SPME) and coupled gas chromatography – mass spectrometry (GC-MS), where the most abundant chemicals were α-pinene, trimethylindan and cyclohexylbenzene. These compounds have been previously reported in other plant species, including some species considered as host plants for Melolonthidae.

Key words: Attraction, plant volatiles, “rose chafer”, SPME, GC-MS.

Resumen

Los productos o substancias volátiles de plantas median las interacciones entre los organismos de diferentes niveles tróficos, incluidos los insectos fitófagos. Estas interacciones se han citado en un contexto químico-ecológico para especies de Coleoptera Melolonthidae. Sin embargo, no hay informes previos sobre estas interacciones entre las especies de Melolonthidae distribuidas en México y sus plantas hospederas. Por ello, se registró la interacción entre adultos de *Macrodactylus nigripes*

Bates (Coleoptera:Melolonthidae) y el arbusto de azumiate (*Baccharis salicifolia* Ruíz and Pav.) Pers. (Asteraceae) y se extrajeron e identificaron los compuestos volátiles de las hojas que podrían estar involucrados en la atracción de estos insectos. Se describió la secuencia de patrones de comportamiento que conforman esta interacción. Se observó que las hembras de *M. nigripes* emergen del suelo y se desplazan en vuelo hacia las hojas de los arbustos de azumiate. Cada hembra se posiciona en la hoja y colocan sus mandíbulas en el margen de éstas y comienzan a moverlas de derecha a izquierda para obtener pequeños fragmentos de hojas e ingerirlos. Asimismo, las hembras muestran un patrón de comportamiento de 'llamado sexual'. Enseguida, los machos emergen del suelo y repiten el comportamiento de las hembras, acercándose a ellas para la cópula. Se generó un listado de los compuestos volátiles de las hojas de azumiate mediante microextracción en fase sólida (SPME, por sus siglas en inglés) y cromatografía de gases acoplada a espectrometría de masas (CG-EM), donde los compuestos más abundantes fueron α-pineno, trimetilindan y ciclohexilbenceno. Estos compuestos se han reportado anteriormente en otras especies de plantas, incluidas algunas especies consideradas como plantas hospederas para integrantes de Melolonthidae.

Palabras clave: Atracción, volátiles de plantas, "escarabajo de las rosas", SPME, GC-MS.

Introduction

Plant volatiles are infochemicals based on complex mixtures of a wide variety of organic compounds. These volatiles have important ecological functions for plants such as pollinators' attraction, seed dispersal and resistance to predators and pathogens. Also, they can mediate interactions between organisms such as phytophagous insects and their parasitoids and predators (Knolhoff and Heckel, 2014). These interactions have been cited in a chemical-ecology context for species of Melolonthidae (Coleoptera) and the attraction of adults to plant volatiles has been studied (Loughrin et al., 1995; Reinecke et al., 2002). The adults of *Macrodactylus* sp. ('rose chafers') are beetles of Melolonthidae and feed on leaves, severely damaging flowers and fruits. They are pest of many species such as roses (*Rosa* spp.), grapes (*Vitis* spp.), apples (*Malus* spp.), cherries (*Prunus* spp.), strawberries (*Fragaria* spp.) and many other trees, shrubs and cultivated plants (Williams et al., 1990; Arce-Pérez and Morón, 2000). There are many wild and cultivated plants that are visited *M. nigripes* adults, unfortunately, they are only mentioned as *Macrodactylus* spp., 'rose chafers', 'frailecillos' or 'taches', revealing the lack of taxonomic knowledge. Inversely, *Macrodactylus* species are only mentioned on leaves, on grasses, on flowers or without information of capture. About 66 species of plants in 26 families, have been reported to function as alimentation, aggregation sites and/or reproduction sites for *Macrodactylus* adults. The families of host plants for *Macrodactylus* are Gramineae (50%), Rosaceae (42.30%), Leguminosae (30.76%), Asteraceae and Lauraceae (15.38%), and Pinaceae (11.53%) (Arce-Pérez and Morón, 2000). For Asteraceae, 'seep willow' shrubs are included in *Baccharis* genus, which is represented by 340 species distributed in Brazil, Argentina, Colombia, Chile, and Mexico (Heiden et al., 2012). In the chemical ecology context, there are only three reports in which the plant volatiles of *Baccharis salicifolia* have been studied obtaining by distillation its essential oils (Loayza et al., 1995; Malizia et al., 2005; Carrizo et al., 2009). There are no previous studies about the volatiles of species of seep willows distributed in Mexico.

In the present study, we generated information about the interaction between *B. salicifolia* and *M. nigripes*, an agricultural important species in Mexico (Arce-Pérez and Morón, 2000; Guzmán-Mendoza et al., 2016). In particular, we recorded in field the behavioral responses of *M. nigripes* Bates in presence of seep willows shrubs and we obtained the chemical profile of the leaves of these by solid phase microextraction (SPME) and coupled gas chromatography – mass spectrometry (GC-MS) techniques.

Materials and methods

Study site

The records of the interaction between adults of *M. nigripes* and seep willow shrubs were taken in a semi-disturbed area adjacent to maize crops, located in the municipality of San Pablo del Monte, Tlaxcala (19° 07' 00" N and 98° 10' 00" O).

Observational records

Behavior records were performed by means of direct observation (focal and continuous records) (Altmann, 1984). We conducted a total of twenty-four observational sessions, twelve for females (n = 12) and twelve for males (n = 12). Each session lasted 90 minutes and was carried out from 2012 to 2015 between 11:00 and 13:30 hours. We recorded and described the movements, actions and positions of adult females and males in twenty-four seep willows shrubs randomly chosen. Each description begins when the beetles emerges from the soil and finishes when they began to cut up the leaves with the mandibles.

Data analysis

The sequences of behavioral patterns obtained of the interaction between rose chafers and seep willow were measured and flow charts of these sequences were developed. The p*ercentage* of times that a particular action pattern follows another (given the first action pattern), and transitions duration were calculated. The principal behavior patterns were described in an ethogram and patterns-transitions sequences were represented in a kinematic diagram.

Leaves volatiles extraction and identification

A directed sampling was conducted to obtain leaves of the same size and healthy appearance from seep willow shrubs. For each extraction, six leaves with same size and weight were introduced on filter-paper at the bottom of the SPME recipient. Polydimethylsiloxane/Divinylbenzene (PDMS/DVB, 65 μm) fiber coating was used. The holder with the PDMS/DVB fiber coating was placed on the entrance of the upper section for 60 min, capturing the leaves volatiles. We performed a blank experiment for each extraction. For each of the leaves and blank experiments, we performed ten repetitions (n = 10).

The volatiles isolated by SPME were identified in a coupled-gas chromatography 7890B coupled with a high-resolution mass spectrometer Agilent 7200 (Q-TOF), equipped with a HP-5MS 5% phenyl-methyl-siloxane column, of 30m diameter x 0.25μm of inner diameter. The conditions during this analysis were: 250 °C for the injector temperature, splitless injection mode and helium 1.2 ml/min as the carrying gas. The initial temperature was of 50 °C; it was maintained for 5 minutes until it reached 300 °C at a speed of 10 °C/min.

A spectral comparison of the chromatogram peaks was used to identify the volatile constituents of the leaves. In the mass detector we used chemical ionization with methane to produce ions that were assigned to different chromatogram peaks. Based on the proposed chemical structures from *Pherobase* database and specialized articles, we calculated de exact mass of the detected compounds and the mass/charge difference (Δm/z) and chemical structures were proposed.

Results

Behavioral records

The behavioral patterns shown by females of *M. nigripes* are shown in Table 1, as well as patterns and transitions sequences are presented in Figure 1. Females emerge from the soil at 11:00 hours and travel in short flights to seep willow shrubs. This movement lasts, on average, 240 secs. Each female settle on the leaf and moves forward and backward for 7 secs, stopping in the superior or posterior leaf margin, positioning itself opposite to the soil and hanging with their legs. Right away, they start moving their abdomen in circles (rotates around its own axis), lifting it in a 45° angle and periodically moving up and down their metatarsus, constantly rubbing them during short periods of time. It has been suggested that this behavior corresponds to the 'calling' in this species (Romero-López et al., 2010). In this position or settling on the leaf margin, the females put their mandibles on the leaf margin and starts moving them from right to left for 82 secs obtaining small leaf fragments to ingest. These mandibles movements could be the feeding behavior of *M. nigripes*. Males emerge from the soil 30 seconds after the females repeating the females' behavior, except for the abdomen movement.

Leaves volatiles identification

We obtained fifteen chromatogram peaks from which ten were related to chemical compounds structures. Each chromatogram peak with its retention time (RT), molecular ion exact mass, error values (Δm/z) and proposed structure are shown in Table 2. Peak 10 with a retention time of 16.82 minutes was the most intensive peak. We found peaks b1, b2, b3 and b4 also in the blank (Table 2, Figure 2).

Discussion

This is the first study that describes at the same time the feeding behavior and the plant-insect interaction of any Melolonthidae species. Previous studies have only reported these aspects separately. For sexual activity, Romero-López et al. (2007) provided a brief description of the pre-mating behavior of four *Phyllophaga* species on the leaves of *Quercus* sp. Also, has been described the pre-mating behavior of *Phyllophaga obsoleta* Blanchard and some interactions between the adults of this species and leaves of *Bouganvillea* sp. (Romero-López and Arzuffi, 2010). However, both studies do not provide details of the plant-insect interaction. Another studies, tested the preference of *M. nigripes* adults for different chemical stimuli, including *B. salicifolia* leaves using a portable olfactometer (Nieves-Silva and Romero-López, 2016). For plant-insect interactions, Ruther et al. (2002) demonstrated that the volatiles of the host plant of *Melolontha hippocastani* F. combined with a sex pheromone function as attractants of adults of this species. *Baccharis conferta* (Kunth) has been reported as a host plant of *M. nigripes* (Arce-Pérez and Morón, 2000). It is well known that plant species from the same family and genus share about 45-50% of their chemicals (Bottia et al., 2007; Vazquez et al., 2007).

For other hand, in this study has been recorded the presence of cumene in *B. salicifolia* leaves. This chemical compound has also been reported for *Baccharis dentata* (Vell.) (Xavier et al., 2012). The chemicals calamene, α-pinene and β-pinene have been previously described for *B. salicifolia* using the hydrodistillation method (Loayza et al., 1995; Malizia et al., 2005; Carrizo et al., 2009; Budel et *al.*, 2018). The use of different techniques to obtain volatile compounds influence in their detection, however, also influence the biotic and abiotic factors and even the sex of the plant (Drijfhout, 2010; Zuccolotto et al., 2019). The hydrodistillation technique has been used for the extraction of compounds of the genus *Baccharis* for medicinal purposes, but this technique can only extract volatiles carried by water vapor (Salomé-Abarca et al., 2015).

In this study we used the SPME which has been reported as the appropriate method for volatile compounds extraction in chemical ecology (Drijfhout, 2010). We also report the presence of phenylmethanol in *B. salicifolia* leaves. This compound has been documented as an attractant of the Melolonthidae species *Holotrichia oblita* Faldermann, *Holotrichia parallela* Motschulsky, *Maladera orientalis* Motschulsky, *Anomala corpulenta* Motschulsky, *Anomala octiescostata* Burmeister and *Popillia guadriguttata* F. (El-Sayed, 2019). Future studies should be focused in proving the biological activity of the phenylmethanol as an attractant of *M. nigripes* and other rose chafer species.

Conclusions

Three principal behavior patterns of females and males of *M. nigripes* interactions with leaves of seep willow were observed: directed flight, settling and mandibles movements to cut leaves, and concurrently the females display a similar behavioral sequence to calling. The most abundant volatile compounds such as trimethylindan, α-pinene, ciclohexylbenzene, as well as phenylmethanol previously reported, could have an important role in the attraction of *M. nigripes.*

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References

Altmann, J. 1984. Observational sampling methods for insect behavioural ecology. Florida Entomologist. 67:50-55. DOI:10.2307/3494104.

Arce-Pérez, R. and Morón, M. A. 2000. Taxonomía y distribución de las especies de *Macrodactylus Latreille* Coleoptera:Melolonthidae) en México y Estados Unidos de América. Acta Zoológica Mexicana 79:123-239.

Bottia, E. J., Díaz, O. L., Mendivelso, D. L., Martínez, J. R. and E. E. Stashenko. 2007. Comparación de la composición química de los metabolitos secundarios volátiles de cuatro plantas de la familia Piperaceae obtenidos por destilación-extracción simultánea. Scientia et Technica 8(33):193-195. http://dx.doi.org/10.22517/23447214.6167.

Budel, J. M.; Wang, M.; Raman, V.; Zhao, J.; Khan S. I.; Rehman, J. U.; Techen, N.; Tekwani, B.; Monteiro, L. M.; Heiden, G.; Takeda, I. J. M.; Farago, P. V. and Khan, I. A. 2018. Essential oils of five *Baccharis* species:investigations on the chemical composition and biological activities. *Molecules*, 23(10):2620. https://doi.org/10.3390/molecules23102620.

Carrizo, R.; Ponzi, M.; Ardanaz, C.; Tonn, C. E. and Donadel, O. J. 2009. Chemical composition of essential oil of *Baccharis salicifolia* Ruiz and Pavon) Pers. and antibacterial activity. Journal of the Chilean Chemical Society 54(4):475-476. http://dx.doi.org/10.4067/S0717-97072009000400034.

Drijfhout, F. 2010. Chemical Ecology. In:eLS. John Wiley and Sons, Ltd:Chichester. https://doi.org/10.1002/9780470015902.a0003265.pub2.

El-Sayed, A. M. 2019. The Pherobase:Database of Pheromones and Semiochemicals. http://www.pherobase.com.

Gonzaga, L.; Costa, I. and Geraldo M. 2005. Género *Baccharis* Asteraceae):Aspectos químicos, económicos y biológicos. *Química nova,* 28(1):85-94. http://dx.doi.org/10.1590/S0100-40422005000100017.

Guzmán-Mendoza, R.; Salas-Araiza M. D.; Calzontzi-Marín J.; Martínez-Yáñez R. and Pérez-Moreno L. 2016. Efectos de la fertilización en cultivos de maíz sobre la abundancia y distribución de *Macrodactylus nigripes* Coleóptera:Malolonthidae)

de las tierras altas del centro de México. Acta Universitaria 26(1):3-11. http://dx.doi.org/10.15174/au.2016.802.
Heiden, G.; Baumgratz, J. F. A. and Esteves, R. L. 2012. *Baccharis* subge. Molina Asteraceae) no estado do Rio de Janeiro, Brasil. Rodriguésia, 63(3):649-687. https://dx.doi.org/10.1590/S2175-78602012000300013.
Knolhoff, L. M. and Heckel, D. G. 2014. Behavioral assays for studies of host plant choice and adaptation in herbivorous insects. Annual Review of Entomology 59:263-278. DOI:10.1146/annurev-ento-011613-161945.
Loayza, I.; Abujder, D.; Aranda, R.; Jakupovic, J.; Collin, G.; Deslauriers, H. and Jean, F. 1995. Essential oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia.* Phytochemistry 38(2):381-389. https://doi.org/10.1016/0031-9422(94)00628-7.
Loughrin, J. H.; Potter, D. A. and Hamilton-Kemp, T. R. 1995. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle *Popillia japonica* Newman. Journal of Chemical Ecology 21(10):1457-1467. DOI:10.1007/BF02035145.
Malizia, R. A.; Cardell, A.; Molli. J. S.; González, S.; Guerra, P. E. and Grau R. J. 2005. Volatile constituents of leaf oils from the genus *Baccharis*. Part II:*Baccharis obovata* Hokker et Arnott and *B. salicifolia* Ruiz et Pav.) pers. Species from Argentina. Journal of Essential Oil Research 17(2):194-197. https://doi.org/10.1080/10412905.2005.9698873.
Nieves-Silva, E. and Romero-López, A. A. 2016. Olfatómetro portátil para el estudio de interacciones en frailecillos Coleoptera:Melolonthidae) y plantas. Entomología Mexicana 3:516-522. http://www.socmexent.org/entomologia/revista/2016/EC/Em%20516-522.pdf.
Reinecke, A.; Ruther, J. and Hilker, M. 2002. The scent of food and defense:green leaf volatiles and toluquinone as sex attractant mediate mate finding in the European cockchafer *Melolontha melolontha.* Ecology Letters 5(2):257-263. https://doi.org/10.1046/j.1461-0248.2002.00318.x.
Romero-López, A. A. and Arzuffi, R. 2010. Evidencias sobre la producción y liberación de compuestos bioactivos de un melolóntido mexicano. En L. A. Rodríguez del Bosque y M. A. Morón Eds.). Ecología y Control de Plagas Edafícolas.. Publicación especial del Instituto de Ecología A.C. pp. 203-222. México. http://bibliotecasibe.ecosur.mx/sibe/book/000049490.
Romero-López, A. A.; Arzuffi, R. and Morón, M. A. Eds.. 2010. Plagas del suelo. Editorial Mundi-Prensa, México.
Romero-López, A.A.; Aragón, A. and Arzuffi, R. 2007. Estudio comparativo del comportamiento sexual de cuatro especies de *Phyllophaga* Coleoptera:Melolonthidae. En E. G. Estrada, A. Equihua, C. Luna, J.L. Rosas-Acevedo Eds.). Sociedad Mexicana de Entomología. Entomología mexicana 6:275-281.
Ruther, J.; Reinecke, A. and Hilker, M. 2002. Plant volatiles in the sexual communication of *Melolontha hippocastani*:response towards time dependent bouquets and novel function of Z)-3-hexen-1-ol as a sexual kairomone. Ecological Entomology 27(1):76. https://doi.org/10.1046/j.1365-2311.2002.0373a.x.
Ruther, J.; Reinecke, A.; Thiemann, K.; Tolasch, T.; Francke, W. and Hilker, M. 2000. Mate finding in the forest cockchafer *Melolontha hippocastani*, mediated by volatiles from plants and females. Physiological Entomology 25(2):172-179. https://doi.org/10.1046/j.1365-3032.2000.00183.x.
Salomé-Abarca, L. F.; Soto-Hernández, R. M.; Cruz-Huerta, N. and González-Hernández, V. A. 2015. Chemical composition of scented extracts obtained from *Calendula officinalis* by three extraction methods. Botanical Siciences 93(3):633-638. http://dx.doi.org/10.17129/botsci.143.
Vazquez, A. M.; Goleniowski, M.; Brunetti, P.; Cantero, J. J.; Demmel, M. G.; Criado, S.; Ferrari, M. C. and Aimar, M. L. 2007. Estudio comparativo de la composición química compuestos orgánicos volátiles) por HS-SPME/GC-MS de *Hedeoma multiflora* Benth. Lamiaceae), micropropagadas y de poblaciones

silvestres. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 6(5):284-285. https://www.redalyc.org/pdf/856/85617508076.pdf.

Williams, R. N.; McGovern, T. P.; Klein, M. G. and Fickle, D. S. 1990. Rose chafer Coleoptera:Scarabaeidae):improved attractants for adults. Journal of Economic Entomology 83:11-116. https://doi.org/10.1093/jee/83.1.111.

Xavier, V. B.; Vargas, R. M. F.; Cassel, E.; Lucas, A. M.; Santos, M. A.; Mondin, C. A.; Santarem, E. R.; Astarita, L. V. and Sartor, T. 2012. Mathematical modeling for extraction of essential oil from *Baccharis* spp. by steam distillation. Industrial Crops and Products 33(3):599-604. https://doi.org/10.1080/0972060X.2012.10644129.

Zuccolotto, T.; Bressan, J.; Lourenco, A. V. F.; Bruginski, E.; Veiga, A.; Marinho, J. V.N.; Raeski, P. A.; Heiden, G.; Salvador, M. J.; Murakami, F. S.; Budel, J. M. and Campos, F. R. 2019. Chemical, antioxidant, and antimicrobial evaluation of essential oils and anatomical study of the aerials parts from Baccharis species Asteracea. Chemistry AND Biodiversity 16(4. https://doi.org/10.1002/cbdv.201800547.

Table 1. Description of the basic body movements and behavior patterns of females and males of *Macrodactylus nigripes*.

Behavior pattern (principal sequence)	**Description**
Fligth	Direct air displacement towards the leaves of the plant with periodic and constant movements of wings.
Settling on the leaf and recognition	First contact with the leaf. The beetle puts pro-, meso- and metatarsus, as well as the antennae, on the leaf margin.
Movements of mandibles to cut the leaf	Movements of mandibles of right side to left in the opposite direction, cutting the leaf in small fragments
Secondary sequence	
Not directed flight	Air random displacements towards anywhere but leaves
Not movements of mandibles	The mandibles remain immobile. There's no physical contact between mandibles and leaf
Precopulatory behavior	
Calling	The females display abdominal bend and abdominal movements in circles lifting it in 45° angle and periodically moving up and down their metatarsus

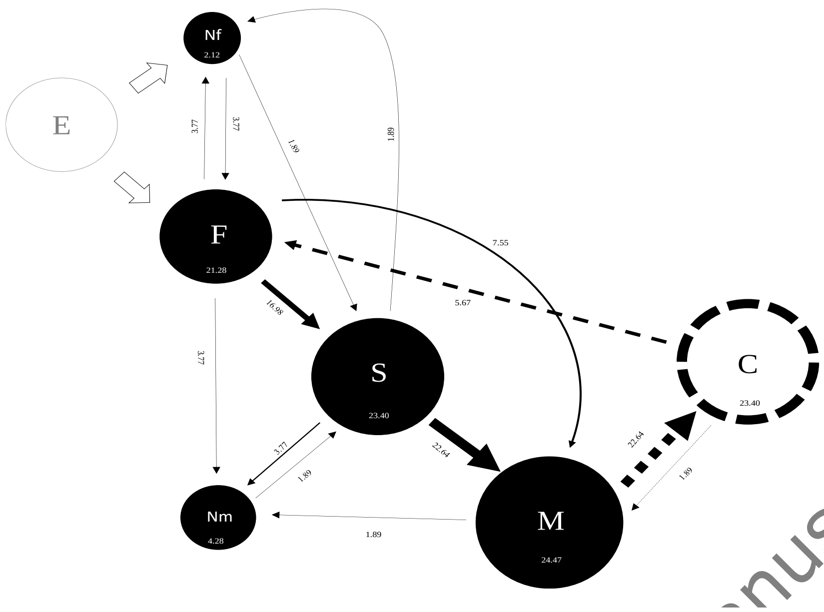

Figure 1. Kinematic diagram of the interactions between females and males of *Macrodactylus nigripes* and seep willow plants in field observations.

Circles and arrows represent the behavior patterns and transitions between behavior patterns, respectively. The numbers into circles and the numbers associated with arrows represent observed frecuencies (in percentages) of successive behavior patterns and transitions of a complex behavioral sequence. The size of the circles is proportional to the relative frequency of each behavior pattern. The width of the arrow is proportional to the relative frequency of transition.

F= Fligth; S= Settling on the leaf and recognition; M= Movements of mandibles to cut the leaf; Nf= Not directed flight; Nm= Not movements of mandibles; C= Calling. White circle= Emergency of females and males from soil / Black circles= Principal behavioral sequence/Dotted line circle= Sexual behavior pattern / White arrow= Transitions after the emergency of females and males from soil/Continue line arrows= Principal transitions behavioral sequence/Dotted line arrows= Connection between interaction plan-insect and sexual behavior transitions.

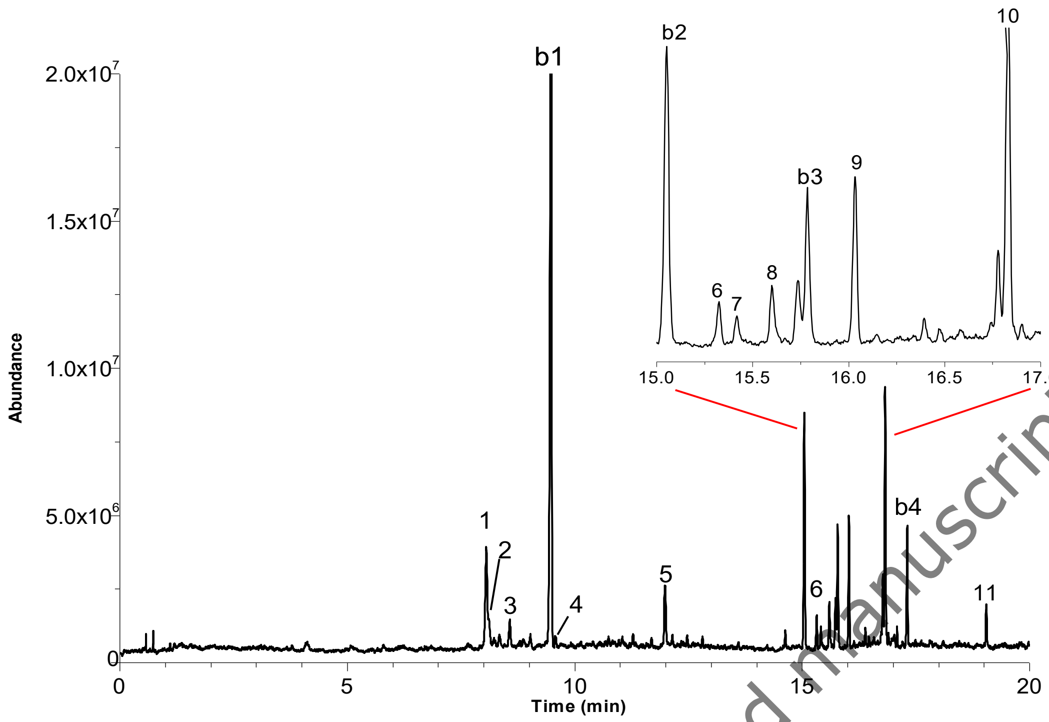

Figure 2. Chromatogram of the volatiles of *Baccharis salicifolia* leaves extracted using the SPME and GC-MS techniques.

Table 2. Proposed structures for the extracted volatiles of seep willow leaves by SPME and CG-MS.

Volatile compounds	PN	RT (min)	Formula	Experimental mass	Exact mass	Δm/z
α-pinene	1	8.06	$C_{10}H_{16}$	136.1252	136.1252	0
β-pinene	2	8.12	$C_{10}H_{16}$	136.1252	136.1252	0
1,2,3-trimethylbenzene 2,4- trimethylbenzene 1,3,5- trimethylbenzene cumene	3	8.58	C_9H_{12}	120.0938	120.0939	-0.83
phenylmethanol	4	9.59	C_7H_8O	108.0575	108.0575	0
2- phenyl-propenal	5	11.99	C_9H_8O	132.0574	132.0575	-0.75
ethylidenecyclohexane	6	15.32	C_8H_{14}	110.1095	110.1096	-0.90
trans-calamenene	7	15.42	$C_{15}H_{22}$	202.1711	202.1721	-4.94
	8	15.60				
unidentified	9	16.03		95.0856		
trimethylindan 1,1,4- trimethylindan 1,1,5- trimethylindan 1,1,6- trimethylindan ciclohexylbenzene	10	16.82	$C_{12}H_{16}$	160.1246	160.1252	-3.74
styrene	11	19.04	C_8H_8	104.0626	104.0626	0

PN= peak number; RT= retention time; Δm/z= error values.