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Volatiles induction in response to mechanical damage is reduced by domestication in murtilla

[La inducción de volátiles en respuesta al daño mecánico es reducida por la domesticación en murtilla]

Manuel Chacón-Fuentes[1,2,3], Leonardo Bardehle[2,3,4], Ivette Seguel[5], Cristian Medina[1,2,3] & Andrés Quiroz[2,3]

[1]*Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile*
[2]*Lab. de Química Ecológica, Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile*
[3]*Centro de Investigación Biotecnológica Aplicada al Medio Ambiente (CIBAMA), Universidad de La Frontera, Temuco, Chile*
[4]*Centro de Fruticultura, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile*
[5]*Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación Carillanca, Temuco, Chile*
Contactos | Contacts: **Andrés QUIROZ** - ***E-mail address:*** **andres.quiroz@ufrontera.cl**

Abstract: Volatiles compounds are involved in defensive induction against insects, playing an important role in insect-plant interaction being induced by response to mechanical damage. However, they could decrease according to the domestication degree in cultivated plants. Currently, it has been established that secondary metabolites are reduced due to the domestication process in murtilla. Hence, the follow question emerges: Are volatile organic compounds induced by mechanical damage reduced in cultivated murtilla plants in relation to wild plants? Two cultivated ecotypes and their respective wild counterparts were sampled. Volatiles compounds were obtained using Porapak-Q columns and analyzed by gas chromatography. Results showed that compounds as 2-hexanone, α-pinene, 2-thujene, 3-thujene and 1,8-cineole were more abundant in wild plants exposed to a mechanical damage than cultivated plants. Hence, these compounds have been associated to induced defense, these results suggest that domestication reduced the induction of defensive volatiles in cultivated murtilla in response to mechanical damage.

Keywords: Cultivated; Wild; Native; Ecotypes; Induce defense; *Ugni molinae*

Resumen: Los compuestos volátiles están implicados en la defensa inducida contra insectos, desempeñando un papel importante en esta interacción. Sin embargo, estos compuestos podrían disminuir según el grado de domesticación. Actualmente, se ha reportado que algunos metabolitos secundarios son reducidos en plantas de murtilla domesticadas. Por lo tanto, surge la siguiente pregunta de investigación: ¿Los compuestos orgánicos volátiles inducidos por el daño mecánico son reducidos en plantas cultivadas de murtilla en comparación con plantas silvestres? Para dos ecotipos cultivados y sus respectivas contrapartes silvestres, los compuestos volátiles fueron capturados usando columnas de Porapak-Q y las muestras analizadas por cromatografía gaseosa. Los resultados mostraron que compuestos tales como 2-hexanona, α-pineno, 2-tujeno, 3-tujeno y 1,8-cineol fueron más abundantes en plantas silvestres expuestas a daño mecánico que en cultivadas. Debido a que estos compuestos se han asociado a defensa inducida, estos resultados sugieren que la domesticación reduce la inducción de volátiles en plantas cultivadas sometidas a daño mecánico.

Palabras clave: Cultivada; Silvestre; Nativo; Ecotipos; Defensa inducida; *Ugni molinae*

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INTRODUCTION

The mechanical damage in plants can induce the production of volatile compounds such as green leaf volatiles (GLV´s) or terpenes (Heil, 2009). These volatile compounds play a role in plant defense acting as repellent (Birkett *et al*., 2000) or attractant of predators and parasitoids (DeLange *et al*., 2016), affecting in several ways the trophic interactions (Chen *et al*., 2015). Hence, when plants are damaged mechanically, an induction of defensive volatiles compounds such as GLV´s or terpenes is triggered. Both GLV´s and terpenes are the main type of compounds involved in plant induced defense (War *et al*., 2012). For instance, Rodríguez-Saona *et al*. (2011a) showed that monoterpenes *α*-pinene, limonene, myrcene, linalool and 1,8-cineole and GLV´s such as hexanol, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenyl butyrate increased their concentration in cranberries (*Vaccinium macrocarpon* Ait) plants subjected to a domestication process. In Chile, a native shrub called murtilla (*Ugni molinae* Turcz) has been studied due to their recent domestication process that started around 20 years ago (Seguel *et al*., 2000). There are no reports about the effect of murtilla domestication on the volatiles compounds involved in induced defense. Recent results have shown that this process has resulted in a reduction of secondary metabolites –flavonoids– associated to chemical defenses in murtilla plants (Chacón-Fuentes *et al*., 2015, Chacón-Fuentes *et al*., 2017). The domestication process could be decreasing the production of volatiles compounds previously detected from *U. molinae*, such as *α*-pinene, limonene and 1,8-cineole that are also reported with a repellent activity against insects such as, *Musca domestica* (Diptera: Muscidae), *Tribolium castanaeum* (Coleoptera: Tenebrionidae) and *Myzus persicae* (Hemiptera: Aphididae), acting as plant defense (Tripathi *et al*., 2001, Haselton *et al*., 2015, Jalaei *et al.*, 2015). Rodriguez-Saona *et al.* (2009) reported the induction of 1,8-cineole by mechanical damage in blueberries plants from 30.33 ng/g/h in undamaged plants to 126.54 ng/g/h in damaged plants. Furthermore, the presence of *n*-butyl acetate has also been reported in murtilla plants (Scheuermann *et al*., 2008; Schreckinger *et al.*, 2010) and this C6 compounds could also be increased when wild murtilla plants are exposed to a mechanical damage. Hence, we investigated the domestication effects on the induction of volatile compounds –monoterpenes and C6 compounds– in response to an artificial mechanical damage in wild and cultivated *U. molinae* plants.

MATERIAL AND METHODS

Plant material

The cultivated *U. molinae* ecotypes 08-1 and 12-1 and their respectively wild counterparts collected from Caburgua (39º11`S, 71º49`W) and Pucón (39º17`S, 71º55`W) were established and acclimated for one year at Instituto de Investigaciones Agropecuaria Carillanca, located at La Araucanía region, Temuco, Chile (South of Chile, 38°45`S, 73°21`W). These plants were placed in pots of 8 L capacity to develop a common garden experiment. The substrate consisted in sand (25%) and soil obtained from the same experimental field. Moreover, fertilization was applied on ecotypes and wild plants according to the soil analysis as follow: 80, 44, and 43 g per plant of nitrogen, P_2O_5, and K_2O respectively. Fertilization was separated in 4 periods of application according to the plant phenology in budding (20, 11, 8 g per plant of nitrogen, P_2O_5, and K_2O respectively), flowering (20, 11, 8 g per plant of nitrogen, P_2O_5, and K_2O respectively), ripening (20, 11, 18 g per plant of nitrogen, P_2O_5, and K_2O respectively) and postharvest (20, 11, 8 g per plant of nitrogen, P_2O_5, and K_2O respectively). Finally, these plants were transported to the Laboratorio de Química Ecológica in Universidad de La Frontera (Temuco, Chile) for their volatiles collection (Chacón-Fuentes *et al*., 2015).

Mechanical damage

Mechanical damage was produced cutting the tips of 20 leaves of murtilla with scissors to simulate the area consumed by a chewing larva according to the methodology proposed by Rodríguez-Saona *et al*. (2013). Additionally, mechanical damage generated by a small needle was performed to imitate the damage caused by an ovipositor or an insect stylet, this damage was done five times on the main stem (Piesik *et al*., 2011).

Volatiles collection

Volatiles compounds were collected during 24 h (1 L/min) by enclosing an individual plant into a 900 mL glass chamber (6 cm ID and 30 cm high). Then, were absorbed on 100 mg of Porapak-Q columns (80-100 mesh; Water Associates) previously cleaned with 1 mL of diethyl ether (GC grade; Merck, Darmstadt, Germany) and conditioned at 150ºC for 2 h in nitrogen stream (70 mL/min). The entrainment was performed by using a positive/negative pressure air system according to the methodology proposed by Agelopoulos *et al*. (1999). The air was dried and purified by passage through activated 5-Å molecular sieves and then charcoal, and finally drawn through the glass chamber (Figure No. 1). Volatiles were extracted from the Porapak-Q by elution with 1 mL of hexane (GC-MS grade; Optima Scientific, Darmstadt, Germany), which was concentrated to 100 µL by nitrogen flow.

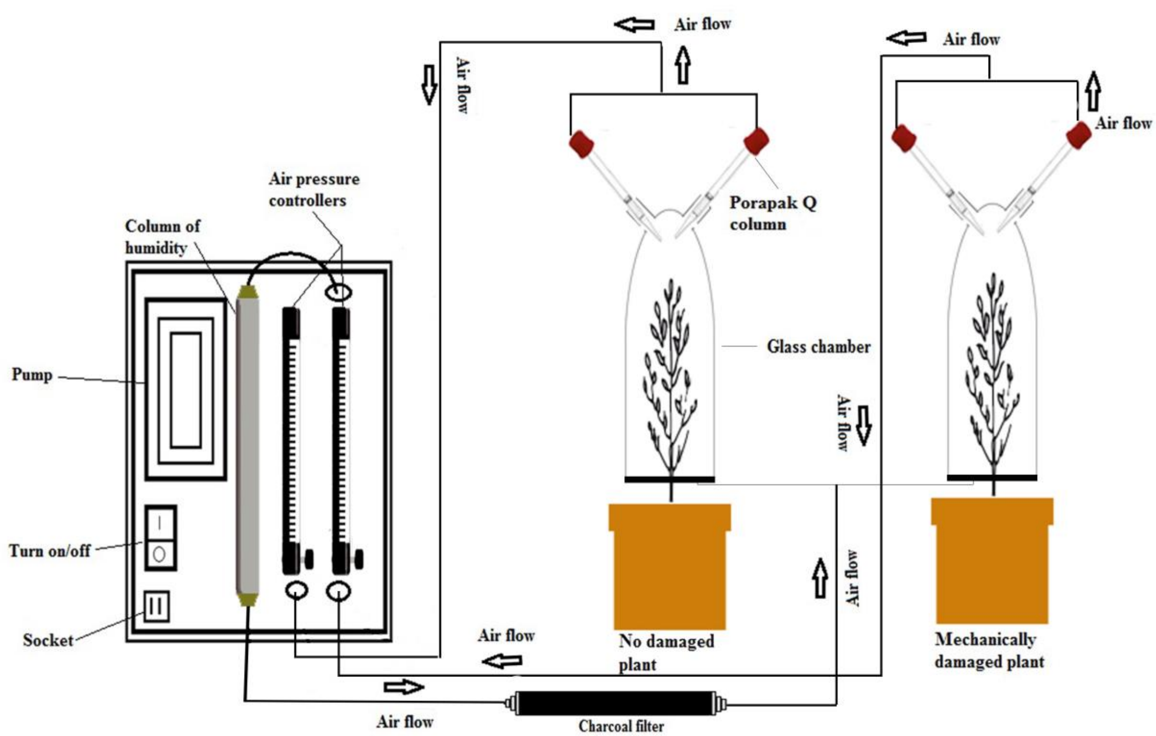

Figure No. 1
Overview of volatiles collection system from with and without damaged murtilla plants
Arrows represent the airflow from the source to porapak Q columns

GC- MS analysis

Volatile compounds were analyzed using gas chromatography coupled to a mass spectrophotometry (GC-MS) (Focus DSQ, Thermo Electron Corporation). Separation was performed using a capillary column BP-1 (30 m x 22 mm x 0.25 *µ*m) and helium gas as carrier (1.0 mL/min) at initial temperature of 40°C for 2 min and increased until 250°C with 5°C of increment/min. Both injector and interface temperatures were kept at 250°C, while the detector temperature was fixed at 200°C. Moreover, the electron impact ionization energy was set up at 70 eV. The acquirement of each mass spectrum was carried out in the mass range from 30 to 350 *m/z*. A total of 1 *µ*L aliquot from both wild and cultivated plants was injected in the GC-MS for volatile compound analysis. A retention time peaks were compared to mass spectrum library NIST (Mass Spectral Library Version 2.0), using a matching algorithm with a reverse search technique to verify highest peaks from the reference compound. Additionally, Kovats Indexes (KI) were determined by injection of alkane series (*C*9 - *C*26). Furthermore, experimental KI's were compared to theoretical KI´s reported in the "NIST" (NIST ver. 2.0, Thermo) database (Babushok *et al*., 2007). All percentages correspond to the relative abundances of each sample.

RESULTS

Results obtained by GC-MS showed a total of six volatiles compounds from *U. molinae* plants, two compounds of six carbons and four monoterpenes (Table No. 1). In general, the treatment (mechanical damage) elicited either the increase of some compounds or elicited the production of new volatile metabolites. 2-hexanone, 3-thujene and 1,8-cineole increased its relative area from 0.40% to 8.92%, 0.33% to 9.90% and 0.39% to 26.84% respectively when Pucón plants were exposed to mechanical damage. Butyl acetate and *α*-pinene were produced only when Pucón plants were injured. Similar results were obtained when plants of ecotype 12-1 were damage. Moreover, *α*-pinene increased from 33.32% to 58.07% after the damage and 1,8-cineole was only released when plants were injured. *α*-Pinene, 2-thujene and 1,8-cineole were emitted from damaged Caburgua plants. Furthermore, *α*-pinene was the unique compound released from murtilla plants belonging to the ecotype 08-1 after the application of the mechanical damage (Table No. 1). Moreover, Table No. 2A and 2B shows the fragmentation pattern for compounds detected by GC/MS. The compounds, 2-hexanone (100 [M^+], 85, 58, 43), 2-thujene (136 [M^+], 121, 107, 93, 79, 77) and 3-thujene (136 [M^+], 121, 105, 93, 91, 77) were for first time recording in *U. molinae* plants subjected to domestication and induced responses (Figure No. 2). Finally, in Pucón undamaged plants was identify the sesquiterpene *β*-caryophyllene (204 [M^+] 133, 93 [BP], 91, 79, 69, 41) represented the 0.57% of the total compounds but, in Pucón plants exposed to a mechanical damage this compound was no detected (Table No. 2A and 2B).

Table No. 1

Compounds name	Pucón no damaged (%)	Pucón MD* (%)	12-1 no damaged (%)	12-1 MD (%)	Caburgua no damaged (%)	Caburgua MD (%)	08-1 no damaged (%)	08-1 MD (%)
C6 compounds								
2-Hexanone	0.40±0.02	8.92±0.15	ND	ND	ND	ND	ND	ND
Butyl acetate	ND	9.31±0.15	ND	ND	ND	ND	ND	ND
Monoterpenes								
α-Pinene	ND	45.00±6.80	33.32±4.33	58.07±41.06	ND	57.13±4.39	ND	100 ± 0.00
3-Thujene	0.35±0.04	9.90±1.02	ND	ND	ND	ND	ND	ND
2-Thujene	ND	ND	ND	ND	ND	11.93±1.43	ND	ND
1,8-Cineole	0.39±0.07	26.84±3.29	ND	41.92±9.64	ND	30.94±1.88	ND	ND
Sesquiterpenes								
β-Caryophyllene	0.57±0.03	ND	ND	ND	ND	ND	ND	ND

*** MD: Mechanical damage; ND: No detected; RT: Retention time**

Table No. 2A

Compounds name	RT (min)	IK exp.[1]	IK lib.[2]
C6 compounds			
2-Hexanone	4.44	-	772
Butyl acetate	5.06	782	799
Monoterpenes			
α-Pinene	8.60	928	934
3-Thujene	8.62	928	926
2-Thujene	9.79	968	968
1,8-Cineole	11.30	1016	1019
Sesquiterpenes			
β-Caryophyllene	22.39	1412	1411

[1] Kovats indices experimental
[2] Kovats indices library

Overall, from Table No. 1 it could be concluded that there is variability within the volatiles produced by wild type of murtilla ecotypes, Pucón lost 2-thujene and 3-thujene and the induction of 2-hexanone when was domesticated to the ecotype 12-1. The α-pinene was induced in Pucón ecotypes when these plants were subjected to a mechanical damage and increased in the domesticated ones. Furthermore, Pucón ecotype elicit more 1,8-cineole due to mechanical damage and in their counterpart this compound was induced when plants were damage mechanically. The ecotype 08-1 elicited a 100% of α-pinene when was subjected to a mechanical damage. However, this ecotype lost the capacity of elicitation of 2-thujene and 1,8-cineole by mechanical damage.

Table No. 2B
Fragmentation pattern of C6 compounds and monoterpenes from *U. molinae* plants analyzed by GC/MS

Volatiles compounds	Fragment (m/z)
C_6 compounds	
2-hexanone	100 [M+], 85, 58, 43 [BP]*
Butyl acetate	116 [M+], 73, 56, 43 [BP]
Monoterpenes	
α-Pinene	136 [M+], 121, 105, 93 [BP], 77
3-Thujene	136 [M+], 121, 105, 93 [BP], 91, 77
2-Thujene	136 [M+], 121, 107, 93 [BP], 79, 77
1,8-Cineole	155 [M+], 139, 108, 93, 81, 71, 43 [BP]
Sesquiterpenes	
β-Caryophyllene	(204 [M+] 133, 93 [BP], 91, 79, 69, 41)

***: Base peak**

DISCUSSION

Plant domestication can modify chemical defenses in several ways (Meyer *et al*., 2012). In this framework, the domestication process affected volatile compounds associated with the induced defense, under natural conditions is expressed only when the plant perceive cues indicating the presence of a potential herbivorous insects (Lundborg *et al*., 2016). In plants, monoterpenes can act as toxins as well as feeding and oviposition deterrents for insects (Litvak & Monson, 1998). For instance, Delphia *et al*. (2007) reported that the total volatiles released from tobacco plants increased from 0 to 288.2 ng/day when plants were mechanically wounding. Moreover, the number of *Heliothis virescens* (Lepidoptera: Noctuidae) adults and larvae per plant were significant decreased (from 5 to 2 and from 65 to 15 insects per plant, respectively) in plants exposed to a mechanical damage. Particularly, *α*-pinene emission rate was significant higher in conifer plants when they were induced by simulated herbivory increasing from 10 in control plants to ~45 μg^{-1} dry mass h^{-1} in simulated herbivory plants (Litvak & Monson, 1998). This research is the first report relating the presence of C6 compounds and monoterpenes subjected to an induced defense and plant domestication. Previous reports by Schreckinger *et al*. (2010) in fruits of *U. molinae* were according to our results, they indicated the presence of volatiles compounds in fruits of murtilla such as butyl acetate, 1,8-cineole and *α*-pinene. Moreover, Scheuermann *et al.* (2008) reported the presence of butyl acetate, *α*-pinene and 1,8-cineole in fruits of *U. molinae*. Results of this research are according to the reported by Rodriguez-Saona *et al*. (2011) who indicated that plant defenses were compromised in cranberries plants modifying the level of monoterpenes as *α*-pinene, 1,8-cineole, limonene and linalool due to induced responses provoked by domestication process. Moreover, the sesquiterpene *β*-caryophyllene- was higher (38.2 ng/3h/plant) in wild maize plants (teosinte) than in cultivated maize plants (6.1 ng/3h/plant) (DeLange *et al*., 2016) and also in blueberries, where plants exposed to a mechanical damage increase the concentration of this compound from 8.85 ng/g/h to 78.77 ng/g/h (Rodriguez-Saona *et al*., 2009). Furthermore, the induction of volatiles compounds by mechanical damage involved in plant defense could alter the insect-plant interactions, affecting the third trophic level generating a decrease in predators or parasitoids associated with biological control. A recent study by DeLange *et al*. (2016) showed that parasitoid *Cotesia marginiventris* (Hymenoptera; Braconidae) was significantly attracted to the odors (mainly terpenes) of the maize ancestor in relation to modern maize. Finally, the C6 compound 2-hexanone and the monoterpenes, 2-thujene and 3-thujene were for first time reported in leaves of *U. molinae* plants subjected to a mechanical damage and domestication process. Overall, our study showed that cultivated ecotypes of murtilla may have lost some of its important signaling capacity for indirect defense. In this sense, domestication is affecting constitutive levels of volatile compounds due to mechanical damage in *U. molinae* plants.

CONCLUSION

Plant domestication affected the releasing of volatile compounds induced by mechanical wounding in cultivated *U. molinae* plants. Monoterpenes and C6 compounds as *α*-pinene, 2-thujene, 3 thujene, 1,8-cineole, 2-hexanone and butyl acetate were induced by mechanical damage in murtilla plants.

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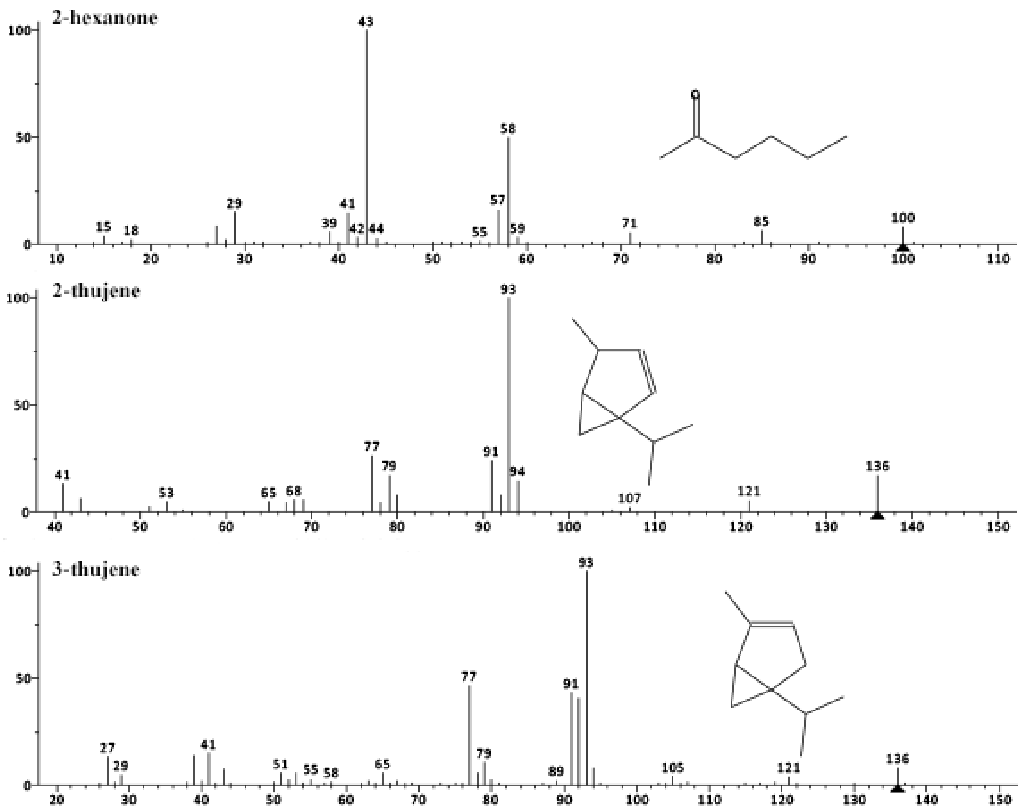
Figure No. 2
Fragmentation pattern of the first record of compounds found in *U. molinae* plants subjected to domestication process and induced responses analyzed by GC/MS



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