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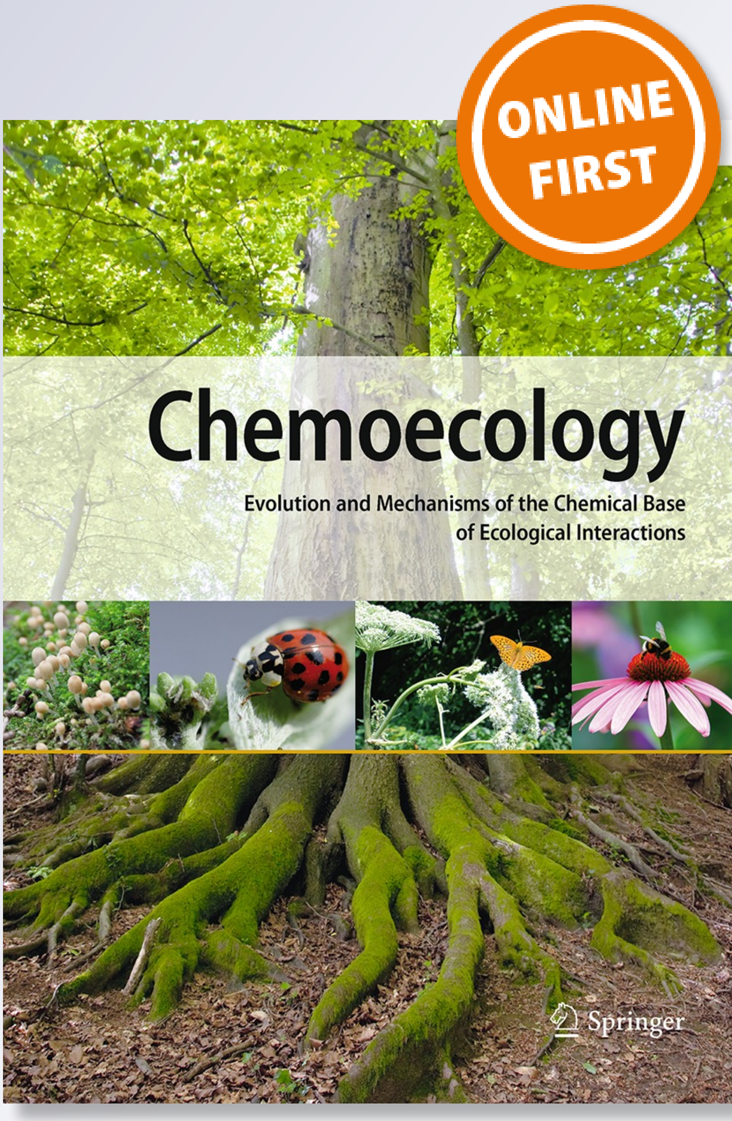

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ORIGINAL ARTICLE

Intraspecific variation of sesquiterpene lactones associated to a latitudinal gradient in *Smallanthus macroscyphus* (Heliantheae: Asteraceae)

María V. Coll Aráoz[1,2] · María I. Mercado[3] · Alfredo Grau[2] · César A. N. Catalán[4]

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Abstract According to theory, variation in plant secondary metabolism against herbivores is driven by variation in biotic and abiotic conditions interacting with plants genotype to determine the expression of resistance traits. Particularly, it has been long postulated that plants growing along latitudinal gradients experience changes in biotic and abiotic interactions, specifically leading to a decrease of plant toxicity towards the poles. We tested this hypothesis using the asteraceous species *Smallanthus macroscyphus*. *Smallanthus* species are known to contain sesquiterpene lactones (STLs), bitter compounds with a broad spectrum of biological activities, including deterrence to herbivores. *S. macroscyphus* showed a decrease in chemical diversity of STLs when investigating populations growing from the tropical regions to less tropical ones. Populations from lower latitudes were found to be more chemically diverse with enhydrin, uvedalin and fluctuanin as main components, while populations southward were chemically fairly uniform, with polymatin A as the main and largely dominant STL. The STL chemistry of *S. macroscyphus* is in agreement with the hypothesis that plants of tropical forests have a greater diversity of secondary metabolites when compared to their temperate counterparts.

Keywords Glandular trichomes · Latitudinal gradient · *Smallanthus* · Sesquiterpene lactones · Plant chemical defenses

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Introduction

It is generally accepted that plants from lower latitudes experience stronger biotic interactions (Coley and Barone 1996; Pennings et al. 2009; Schemske et al. 2009; Salazar and Marquis 2012); therefore, they tend to invest in higher levels of defenses and also in a wider range of structurally related phytochemicals (Coley and Aide 1991; Lewinsohn 1991; Bolser and Hay 1996; Rasmann and Agrawal 2011). Phytochemical diversity could increase the probability of producing biologically active compounds against a larger pool of herbivorous. On the other hand, a complex chemistry that is unpredictable from one population to the next, or from neighboring plants, might be an effective plant defense against specialization by an herbivore (Seaman 1982), since a chemically uniform host species could facilitate the development of specific insect metabolic detoxification systems through the process of specialization.

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A comprehensive recent review, however, surveying five broad groups of plant metabolites (tannins and phenols, flavonoids, alkaloids, resins/oils) pointed out that less than a quarter of studies effectively showed increased production of toxic secondary metabolites at lower latitudes (Moles et al. 2011). Nevertheless, many of the compounds reviewed have roles other than defense, and leaves from lower latitudes may still be better defended than are leaves from higher latitudes, either through possessing a greater range of defenses, greater diversity of related compounds, or through possessing unstudied, taxon-specific defenses.

The genus *Smallanthus* Mackenzie includes at least 21 species, all American, ranging from southern Mexico, Central America and the Andes until northern Argentina. They are herbs, less frequently shrubs or small trees usually associated to disturbed habitats (Grau and Rea 1997). The only member of the genus with economic importance is *Smallanthus sonchifolius,* commonly known as yacon, a traditional Andean crop whose tuberous roots, composed mostly of water, fructooligosaccharides (FOS) and fiber, are consumed raw as a fruit. All the members of the genus *Smallanthus* studied to date yielded melampolide-type sesquiterpene lactones (STLs) (De Pedro et al. 2003; Bach et al. 2007; Mercado et al. 2010; Coll Aráoz et al. 2007). In Asteraceae, STLs are constitutive defenses produced in glandular trichomes of the foliar surface. Apparently, glandular trichomes are fully developed and their secretory activity is concluded at early stages of leaf development; STLs are accumulated underneath the cuticle, where it has been demonstrated that they remain chemically stable during the life cycle of the plant and released only when glandular trichomes are mechanically disrupted (Favi et al. 2008; Mercado et al. 2012). STLs are deterrents to herbivores, toxic to pathogens like fungi and bacteria and serve as allelopathic compounds (Chow and Mullin 1993; Takasugi and Masuda 1996; Ambrósio et al. 2008; Cis et al. 2006).

In a previous work from our group, the leaf chemical diversity of twenty-five accessions of *S. sonchifolius* was explored along a latitudinal gradient from Ecuador to Northwestern Argentina (Mercado et al. 2014). Accessions from Ecuador, Bolivia and Argentina proved to be very chemoconsistent, while quantitative variation in STLs composition was found in accessions from central Peru, the probable region of origin for the species (Grau and Rea 1997). In yacon, the wider distribution of a particular chemotype with enhydrin (**10**) as majoritarian STL, is thought to be due to human selection, not due to natural evolution in the environment, since yacon is a semi-domesticated crop, and was apparently introduced in Ecuador and Argentina, with the Inca conquest (Grau and Rea 1997).

Smallanthus macroscyphus (Baker ex Martius) A. Grau (Heliantheae, Asteraceae) is a wild species from deciduous forests and riverbanks in Southern Bolivia and NW Argentina (Grau and Rea 1997). Like yacon, it possesses tuberous roots, shares the southern area of distribution of the genus and has been considered a putative parental species of this Andean crop (Grau and Rea 1997; Coll Aráoz et al. 2008). Polymatin A (**1**), a 9-hydroxy-8-acyl melampolide with antidiabetic properties (Serra Barcellona et al. 2014), has been reported as the main STL from this species (De Pedro et al. 2003).

Smallanthus macroscyphus frequently dominates the understory of *Juglans australis* stands in the yungas forests. In Argentina, these forests are distributed along a North–South gradient occupying the eastern slopes of fragmented mountain ranges. For this reason, such humid forests are interrupted in their distribution by semiarid areas, especially in Argentina, where they have a high level of fragmentation (Brown et al. 2001). Reduction in biodiversity as the latitude increases has been reported for the Argentinian yungas and has lead to the division of the phytogeographical region in three portions along a latitudinal gradient (De la Sota 1972; Cabrera 1976; Brown et al. 2001; Morales et al. 1995). The more tropical portion of the yungas (typically between 22°00′ and 23°50′S) has a greater diversity of trees, epiphytes, bryophytes, ferns, mammals, amphibia, birds and reptiles (Brown et al. 2001; Grau et al. 2006), and there is emerging evidence that it could be the case of insects too (Navarro et al. 2009). On the other hand, while the changes in species number as the latitude increases have been extensively reported for the yungas, the chemical diversity within a taxon has never been explored to our knowledge.

In the present work, the STL variation from 20 wild populations of *S. macroscyphus* distributed along a North–South latitudinal gradient was studied, and this background was used to discuss the possible causes of the observed chemical variability.

Specifically, we investigated on the occurrence of latitudinal gradients in *S. macroscyphus* chemical defenses concerning both abundance and diversity of sesquiterpene lactones.

Materials and methods

Plant material

Leaves and whole plants of *Smallanthus macroscyphus* (Baker ex Martius) A. Grau from twenty wild populations from the Argentinian–Bolivian border to the south of the province of Tucumán, Argentina (Table 1; Fig. 1) spanning a distance of approximately 600 km were collected.

Table 1 Origin of the populations of *Smallanthus macroscyphus* sampled

Population	Collection site	Latitude	Longitude	Date (day/month/year)	Habitat
1	Nogalar, Salta	22°14	64°42	17/11/06	*Juglans* forest
2	Argentinian–Bolivian border	22°16	64°35	17/11/06	*Juglans* forest
3	San Francisco, Jujuy	23°40	64°55	17/11/06	Transition forest
4	Tiraxi, Jujuy	24°00	65°22	17/11/06	*Juglans* forest
5	Near Tiraxi, Jujuy	24°01	65°17	30/01/10	Side of the road
6	Lozano, Jujuy	24°05	65°24	30/01/10	Side of the road
7	Reyes, Jujuy	24°07	65°24	17/11/06, 16/02/08	*Juglans* forest
8	On the way to Tiraxi, Jujuy	24°07	65°22	30/01/10	*Juglans* forest
9	Zapla, Jujuy	24°14	65°04	17/11/06	*Juglans* forest
10	RN9, Jujuy	24°28	65°17	05/04/07	Riverbank
11	RN9, Salta	24°31	65°22	05/04/07	*Juglans* forest
12	RN9 Alto la Sierra, Salta	24°33	65°23	05/04/07	Side of the road
13	Los Yacones, Salta	24°41	65°28	09/12/06	Side of the road
14	Cámara, Salta	24°55	65°40	25/01/10	With *Mirabilis jalapa*
15	Las Animas, Salta	25°09	65°36	19/01/10	*Juglans* forest
16	Rearte, Tucumán	26°20	65°32	10/11/06, 26/02/09	*Juglans* forest
17	La Junta, Tucumán	26°22	65°31	11/02/08	Riverbanck
18	El Siambon, Tucumán	26°45	65°27	15/11/06	*Juglans* forest
19	Campo de las Azucenas, Tucumán	27°19	65°55	04/11/07	*Juglans* forest
20	On the way to Las Estancias, Tucumán	27°23	65°54	18/11/07	Side of the road

In each case, expanding and mature leaves were sampled. All plant materials were dried at room temperature in the shade. Voucher specimens were deposited in the Herbarium of Fundación Miguel Lillo, city of San Miguel de Tucumán, Argentina.

Smallanthus macroscyphus: Tucumán, Yerba Buena: Horco Molle, CUHM, A. Grau s/n° (LIL 607375). Jujuy, Santa Bárbara, Loc. San Francisco, M.I. Mercado, M.V. Coll Aráoz, G.I. Ponessa s/n° (LIL608677). Jujuy, Dep. El Carmen, RN 9, M.V. Coll Aráoz, M.I. Mercado s/n° (LIL608676). Salta, La Caldera, RN 9, M.V. Coll Aráoz, M.I. Mercado s/n° (LIL608676).

Extracts preparation

The resinous content of capitate glandular trichomes from the leaf surface was extracted from 10 g of dried leaves of each population (5 g of dry leaf from each plant in the case of the studies performed within a population) following the procedure described by Mercado et al. (2010). Briefly, whole air-dried leaves were soaked one by one in chloroform for 20 s at room temperature with a continuous and gentle swinging motion using a stainless steel clip. In every case, the extracts were filtered through a filter paper and the solvent was evaporated under vacuum to yield crude residues, which were dissolved in MeOH (2.5 mL per 100 mg of residue) at 50 °C to facilitate dissolution. After cooling, a 30 % of distilled water (0.75 mL per 100 mg of residue) was added dropwise to precipitate waxes. The hydromethanolic filtrates were evaporated under vacuum to yield dewaxed extracts containing sesquiterpene lactones and diterpenes. Yields are presented in Table 2.

Chemical analysis

The residues were analyzed by GC/MS using a Hewlett-Packard 6890 GC fitted with an Elite-5MS Perkin-Elmer column (5 % phenylmethylsiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) coupled to a 5973 Hewlett-Packard selective mass detector (quadrupole). The following conditions were employed to analyze STLs: injector, GC–MS interphase, ion source and selective mass detector temperatures were maintained at 220, 280, 230 and 150 °C, respectively; ionization energy, 70 eV; injection size: 1 μL (split mode 80:1); carrier gas, helium at a flow rate of 1.2 mL min^{-1}. The oven was programmed as follows: from 180 to 300 °C at 2 °C min^{-1} and then held at 300 °C for 10 min. To be injected, the samples were dissolved in methylene chloride using 25 μL of solvent per mg of dewaxed extract. Percentages are reported as the means of at least three injections and were calculated from the TIC (Total Ion Chromatogram) by the computer.

STLs were identified by their GC retention times and their mass spectra in comparison with pure samples previously isolated in our laboratory (De Pedro et al. 2003; Bach et al. 2007; Mercado et al. 2010; Serra Barcellona

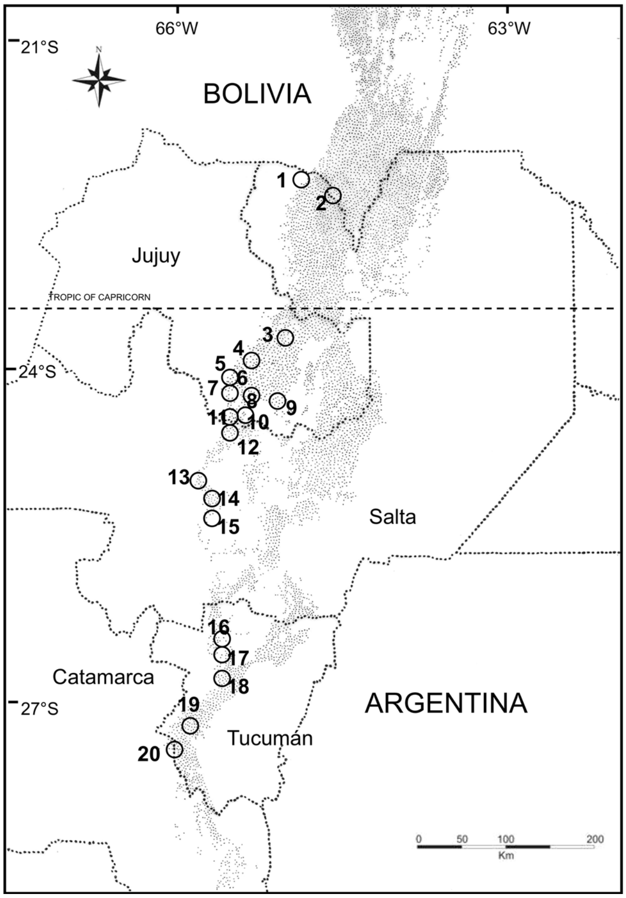


Fig. 1 Geographical location of collection sites of *Smallanthus macroscyphus* (*circles*). The population numbers correspond to those designated in Table 1. Yungas ecoregion is *shaded*

et al. 2014). Additionally, to obtain pure standards and to calculate STL yield, dewaxed extracts from populations of *S. macroscyphus* number 2 and 16 were processed by column chromatography over silica gel, Merck (230–400 mesh, ASTM), using $CHCl_3$ with increasing amounts of EtOAc (10–50 %). Fractions were monitored and grouped on the basis of their TLC profiles (Supplementary material). After solvent evaporation, all the fractions were analyzed by FT-IR. The ones showing γ-lactone carbonyl absorption at 1755–1780 cm^{-1} in their IR spectra were analyzed by GC–MS. Analytical samples of STLs **1**, **2**, **3**, **4**, **5**, **8**, **9** and **10** were obtained by semipreparative RP-HPLC of the fractions obtained by column chromatography using a C8 (octyl) column with MeOH:H_2O (60:40) at a flow rate of 2.0 mL min^{-1} and a differential Refractive Index Detector (injection: 1.2 mL of a solution containing 4.1 mg of STL mixture per mL) and rechromatography on a C18 (octadecyl) column with MeOH:H_2O (1:1) at 1.8 mL min^{-1}, if necessary. The isolated sesquiterpene lactones were identified by MS and ^{1}H, ^{13}C NMR, HMBC and HSQC spectroscopic data.

Table 2 Foliar rinse extracts yields expressed as percentage of dry matter

Population #	Sample weight (gr)	Foliar rinse extract weight (mg)	Dewaxed foliar rinse extract weight (mg)	Yield (%)
1	10	119	89	0.89
2	10	150	92	0.92
3	10	239	185	1.85
4	10	223	153	1.53
5	10	213	145	1.45
6	9.8	327	222	2.27
7	10	177	119	1.19
8	9.2	231	149	1.62
9	10	261	113	1.13
10	10	230	134	1.34
11	10	141	72	0.72
12	10	254	140	1.40
13	10	380	227	2.27
14	9.6	351	264	2.75
15	10	263	180	1.80
16	10	343	247	2.47
17	8.4	244	123	1.46
18	10	219	140	1.40
19	10	178	85	0.85
20	10	170	90	0.90

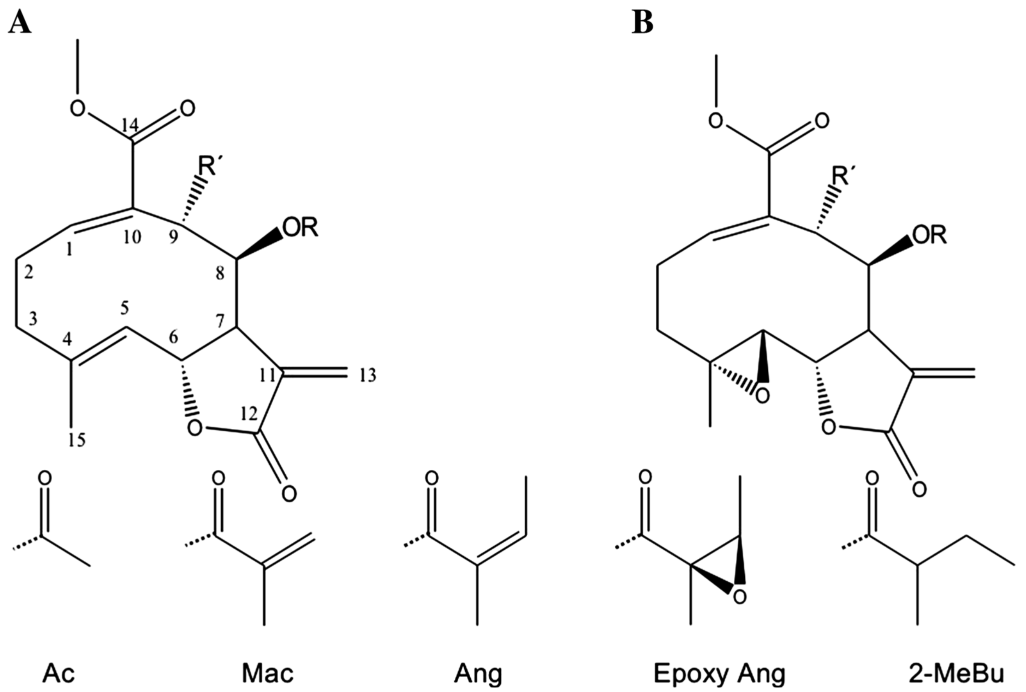
Statistical analysis

Principal component analysis (PCA) was performed to classify and group populations according to STLs composition. A matrix of nine variables and 20 cases was built, considering the presence and amount of STLs in each population. Similarity estimates were analyzed by UPGMA. The evaluation of principal component analysis and similarity coefficient was carried out with the Multivariate Statistical Package V. 3.1 (MSVP) Copyright © 1985–2006 Kovach Computing Services. Distance was estimated by arithmetic averages.

Results

Twenty wild populations of *S. macroscyphus* were sampled (Table 1; Fig. 1). The structures of the STLs identified in the foliar rinse extracts are shown in Fig. 2. Large qualitative differences were found between the populations northward and southward 24°30′S (Table 3). Thus, the studied populations can be grouped into two clusters, cluster 1 spanning populations northward and cluster 2 spanning populations southward 24°30′S.

As can be seen (Table 3; Fig. 3) cluster 1 clearly has a more complex STLs profile, with enhydrin (**10**), uvedalin



Compound		R	R'
1	Polymatin A	Ang	OH
2	Uvedalin	EpoxyAng	OAc
3	Polymatin B	Ang	OAc
4	Isopolymatin A	H	OAng
5		Mac	OH
6	Sonchifolin	Ang	H
7		Mac	H
8		2-MeBu	OAc

Compound		R	R'
9	Fluctuanin	Ang	OAc
10	Enhydrin	EpoxyAng	OAc
11	Polymatin C	Ac	OEpoxyAng
12	Fluctuadin	Mac	OAc

Fig. 2 Melampolides found in *Smallanthus macroscyphus*

(**2**) and fluctuanin (**9**) as the main components of the foliar rinse extracts accompanied by minor amounts of various other STLs such as polymatin B (**3**) polymatin C (**11**), sonchifolin (**6**), fluctuadin (**12**) and **8**. For its part, cluster 2 is characterized by the presence of a single and widely dominant STL, polymatin A (**1**), that accounts for ca. 90 % of the lactone content, accompanied by small amounts of a few other lactones such as isopolymatin A (**4**), **5** and sonchifolin (**6**) but not **9**, **10** or **2** (Table 3; Fig. 3). After three years of growing plants from both clusters in greenhouse conditions, the STL pattern remained unchanged and was found to be essentially identical to those produced by their wild counterparts. Populations from clusters 1 and 2 with different STL patterns were morphologically indistinguishable.

A study of the STL variation within a population was also carried out. Thus, seven individuals from population number 7 (Cluster 1: Reyes, Jujuy), population number 11 (Ruta Nacional 9) and population number 16 (Cluster 2: Rearte, Tucumán) were selected randomly and their leaves analyzed individually (Table 4). Such intra-populational studies showed that, within populations 7 and 11 (cluster 1), relative abundance of STLs varied considerably among individual plants in contrast to population 16 (cluster 2) which showed uniformity, being polymatin A (**1**) the major constituent for specimens I–VII. Polymatin A (**1**) was

Table 3 Percentage of the most relevant sesquiterpene lactones in the analyzed populations of *Smallanthus macroscyphus*

	Latitude	**1**	**2**	**3**	**6**	**9**	**10**	**11**	Others[a]
1	22°16	–	25.8	–	1.0	1.5	57.3	0.7	13.7
2	22°14	2.0	18.8	5.6	0.1	5.1	33.4	11.5	23.5
3	23°40	1.0	35.7	16.8	0.8	5.7	20.6	6.2	13.2
4	24°00	1.7	15.6	4.7	0.3	5.3	55.4	6.4	10.6
5	24°01	2.2	43.2	16.0	–	4.0	4.4	–	30.2
6	24°05	1.5	43.6	13.5	–	5.5	11.7	–	24.2
7	24°07	22.8	17.0	6.4	0.1	2.8	15.2	2.3	33.4
8	24°07	5.2	21.8	9.0	0.1	5.7	18.1	6.0	34.1
9	24°14	1.5	13.6	7.3	1.5	15.3	30.1	21.2	9.5
10	24°28	5.1	4.2	12.1	0.7	37.5	22.5	4.4	13.5
11	24°31	25.8	8.3	8.8	2.1	14.8	27.0	2.7	10.5
12	24°33	80.7	–	–	2.8	–	–	–	16.5
13	24°41	94.4	–	–	–	–	–	–	5.6
14	24°55	87.8	–	–	0.2	–	–	–	12.0
15	25°09	94.6	–	–	0.1	–	–	–	5.3
16	26°20	85.6	–	–	2.8	–	–	–	11.6
17	26°22	93.0	–	–	2.3	–	–	–	4.7
18	26°90	84.2	–	–	2.0	–	–	–	13.8
19	27°19	93.2	–	–	2.3	–	–	–	14.5
20	27°23	92.0	–	–	1.8	–	–	–	7.2

Relative percentages amounts calculated by TIC

[a] Other compounds are: STL **7** for all the populations, STLs **4** and **5** for populations 12–20; STL **8** and **12** for populations 1–11, along with some unidentified STLs

found in both clusters, but while it represents ca. 90 % of the lactone content in cluster 2, it is usually a minor component in cluster 1, even though there were some plants showing a high content of **1** as can be seen in Table 4. It is worth noting that in populations belonging to cluster 1, plants with very different STL patterns grew intermixed, very close to each other.

PCA was conducted using all STLs as variables. Populations 12–20 were grouped together (cluster 2) with a high level of similarity (>90 %), while populations 1–11 were grouped with a 55 % similarity level (cluster 1) (Fig. 4).

In the two populations analyzed by column chromatography, number 2 (Argentinian–Bolivian border) and 16 (Rearte, Tucumán), more than 50 % of the dewaxed extract corresponded to STLs. The other fractions yielded kaurenoic acid and derivatives and smaditerpenic acids, which have been previously reported for *S. sonchifolius* (Mercado et al. 2010).

Discussion

As with most biogeographic hypotheses about variation in biotic interactions and the evolution of plant defense in terms of chemical diversity, we found that lower latitude populations from both species have a more complex STL profile, even though there is no evidence for higher sesquiterpene lactones concentration in these populations (Table 2), as these hypotheses also predict.

In natural populations of *S. macroscyphus*, there are at least two clearly differentiated clusters exhibiting different STL chemistry, one present north of 24°30′S whose individuals have **2**, **9** and **10** as main STL constituents, and the other southward that latitude with **1** as the main and largely dominant STL. Specimens with polymatin A as dominant STL can also be found in the lower latitudes, but in a few individuals within a heterogeneous population, as demonstrated by the intra-populational studies (Table 4). The chemotype in the populations southward 24°30′S is characterized by a very simple STL profile showing a single main component, polymatin A (**1**), accompanied by very few others lactones such as **4**, **5** and **6** which differ only in the nature or position of the acyl residue at C-8 and C-9. Such populations have also proved to be quite uniform; all the individuals within a population had the same profile with little differences in the content of **1** (Table 4). Moreover, we also studied two wild populations of *S. connatus*, one from Misiones province (27°21′S) in the northeastern of Argentina and the other from Buenos Aires (34°48′S) in central Argentina (unpublished data). As in the

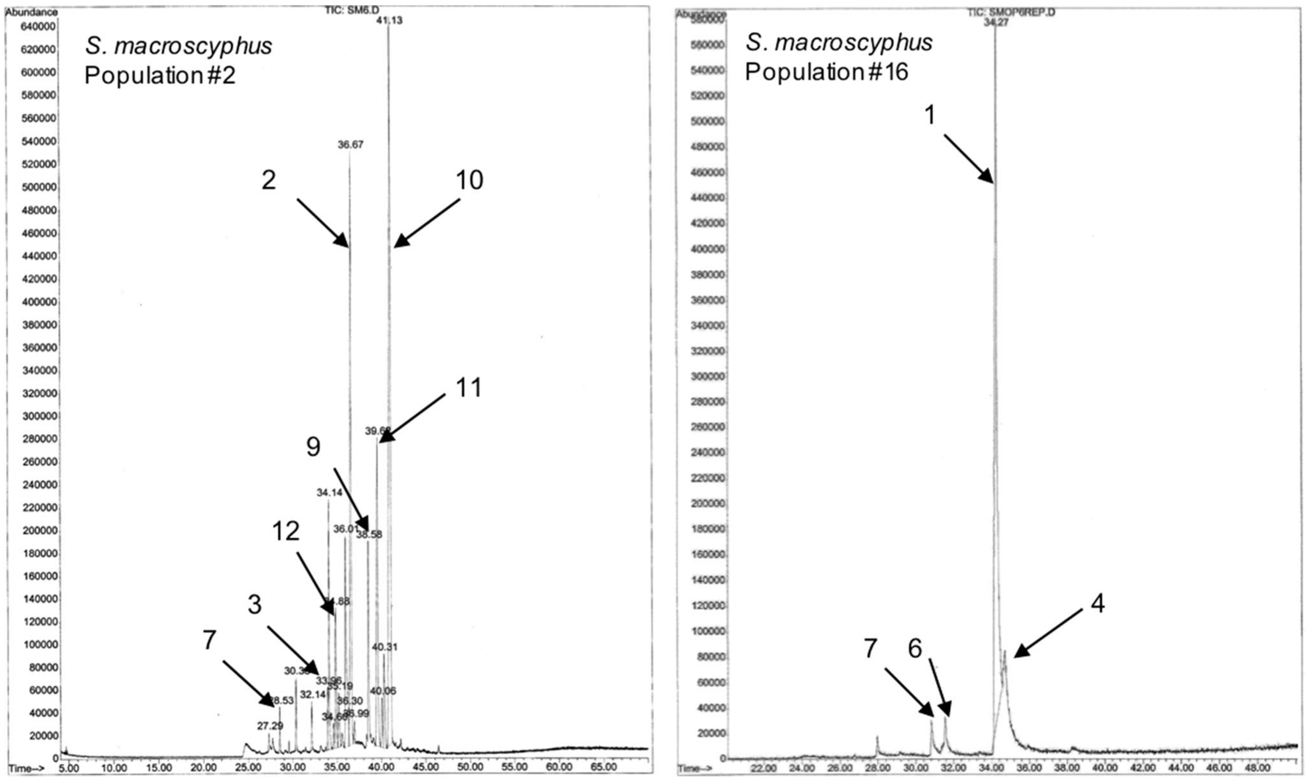


Fig. 3 GC trace of the STL mixture of populations number 2 (**a**) and 16 (**b**) of *Smallanthus macroscyphus*. *Arrows* indicate the main sesquiterpene lactones identified in the foliar rinse extracts

Table 4 Intra-populational variation of sesquiterpene lactones in three different populations of *Smallanthus macroscyphus*

Plant	Population 7 (Reyes, Jujuy)				Population 11 (RN9)				Population 16 (Rearte, Tucumán)			
	1	**2**	**9**	**10**	**1**	**2**	**9**	**10**	**1**	**2**	**9**	**10**
I	5.1	21.1	2.3	17.7	2.5	15.8	3.9	47.4	78.8	–	–	–
II	5.5	33.2	1.4	16.4	48.1	2.7	8.5	28.9	93.8	–	–	–
III	0.5	20.0	7.3	12.6	3.3	–	58.7	–	77.8	–	–	–
IV	50.6	–	2.6	–	73.0	4.2	1.8	3.3	84.5	–	–	–
V	12.9	30.1	1.9	16.8	2.5	21.7	4.3	43.4	89.5	–	–	–
VI	78.2	–	–	–	48.4	–	7.0	36.8	86.0	–	–	–
VII	7.1	14.8	4.4	43.4	3.4	14.0	19.7	29.6	88.9	–	–	–

Relative percentage amounts calculated by TIC

case of *S. macroscyphus*, a more complex chemistry was also found in the tropical population collected in Misiones, that yielded **9**, a closely related dihydroderivative and **1** as majoritarian STLs (accounting for 60.2, 16.0 and 11.3 %, respectively) and other compounds such as polymatin B (**3**) along with several unidentified STLs. For its part, the population of *S. connatus* from Buenos Aires was chemically less complex; the main components were **9** and the dihydroderivative accounting for 86.2 and 13.7 %, respectively, with trace amounts of other lactones.

In comparison to the wild species, *S. sonchifolius* is a very chemoconsistent taxon (Mercado et al. 2014), with poor chemodiversity, since it is a semi-domesticated crop, which is clonally propagated.

All the sesquiterpene lactones identified in the foliar rinse extracts are melampolide type. The different lactones are produced by the presence of a double bond or an epoxide between C-4 and C-5 and the nature of the ester moiety at C-8 and C-9 (Fig. 2). Lactones **9–12** with an epoxy group between C-4 and C-5 have a more oxidized

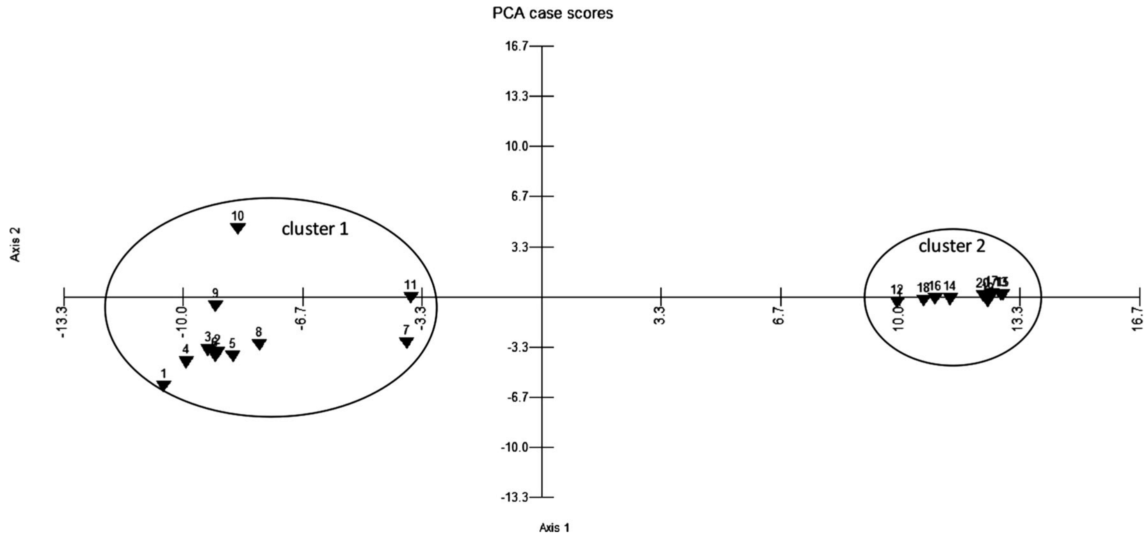


Fig. 4 Principal components analysis of 20 populations of *S. macroscyphus*, based on the STLs content obtained from the leaves

skeleton than lactones **1-5** and **8**, while sonchifolin (**6**) and **7**, with a methylene (–CH_2–) at C-9, are the less oxidized ones. Polymatin A (**1**) could be a precursor for more oxidized lactones such as **9–12** but it is unknown whether the enzymes needed for the oxidation process are absent or inhibited in the southern populations. Loss of biosynthetic capacity (caused by the blocking of a reaction step) is a common event in the Asteraceae (Seaman 1982).

There are two major hypotheses for the production of many different types of closely related secondary compounds by individual plant species. First, increasing the diversity of compounds (especially those that are not costly) may simply increase the probability that one compound would be highly active against a specific consumer (Jones and Firn 1991). This is known as the screening hypothesis because plants produce a large number of metabolites that are subsequently 'screened' by the plant for biological activity. Second, chemical diversity per se enhances plant resistance against the wide variety of organisms interacting with the plant while also attracting predators and parasitoids (Berenbaum et al. 1991; Becerra 2015). Additional to the single compound activity, the production of some chemical mixtures can synergistically improve the activity of compounds when compared with the sum of each individual compound (Berenbaum et al. 1991; Duffey and Stout 1996; Steppuhn and Baldwin 2007; Rasmann and Agrawal 2009).

The origin of the chemical uniformity in the southern populations could be attributed to differential herbivory patterns that may generate through selection more than one adaptive chemical response within the species range (Thompson 2005) or to a limited introduction. The yungas are a fragmented environment, interrupted in their distribution by semiarid areas (Brown et al. 2001), which could hamper gene flow between populations in the north and populations in the south. Interestingly, spatial differentiation of chemotypes occurs at a latitude where most of the biodiversity in terms of species number in the yungas is drastically reduced (Brown et al. 2001; Grau et al. 2006).

It has long been believed that tropical populations are characterized by a greater abundance of chemical traits, both in quantity and diversity, since plant–animal interactions, including herbivory, are thought to be more intense toward the tropics. However, recent syntheses of empirical data have not supported the idea that either herbivory, or plant defenses against herbivores are higher at lower latitudes (Moles et al. 2011, Poore et al. 2012). In this paper, we have not found overall higher level of STLs in the tropical populations, but we did found differences in what concerns diversity of secondary metabolites across latitudinal sites, an idea not tested very often, but recently reviewed by Becerra (2015). Whether this means that *Smallanthus* species from the tropics are better defended and interactions are stronger in the tropics are still unknown.

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Compliance with ethical standards

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