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Phytotoxicity evaluation of sesquiterpene lactones and diterpenes from species of the *Decachaeta*, *Salvia* and *Podachaenium* genera

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ABSTRACT

Decachaeta, *Salvia* and *Podachaenium* genera are known for their wide variety of biological activities. Synthetic herbicides have caused a variety of environmental and resistance problems. Natural products represent an important alternative to combat such issues. Sesquiterpene lactones and diterpenes are families of bioactive natural products for which a range of activities have been described. The bioactivities of nine sesquiterpene lactones, eight heliangolides, one guaianolide and twenty two diterpenes isolated from species of the *Decachaeta*, *Salvia* and *Podachaenium* genera were tested by applying a methodical procedure that involves assays of compounds on etiolated wheat coleoptiles (*Triticum aestivum*), Standard Target Species (STS) and two important weeds (barnyardgrass and brachiaria). The results clearly show that all of the sesquiterpene lactones studied were active on coleoptiles. In addition, six lactones were phytotoxic on both STS and weeds, meaning that these compounds could be used in the development of natural herbicide models.

1. Introduction

The *Decachaeta*, *Salvia* and *Podachaenium* genera are known for their wide variety of biological activities. Extracts of these plants have been described as antimicrobial, antioxidant (Veličković et al., 2011), antiprotozoal, antibacterial (Calzada et al., 2009), anti-mutagenic (Mathew and Thoppil, 2012), antiproliferative (Janicsák et al., 2011) or anti-allergic (Yang et al., 2008) and numerous compounds – including diterpenes and sesquiterpene lactones – with biological activities have been isolated from these plants.

Diterpenes and sesquiterpene lactones are two large families of widely studied natural products that show a broad range of biological activities. For example, diterpenes are known as anti-cancer, anti-diabetic (Nagarajan and Brindha, 2012), anti-inflammatory (Kapewangolo et al., 2015), anti-oxidant (Kolak et al., 2009) or phytotoxic compounds (Carrera et al., 2015). In contrast, sesquiterpene lactones have been reported to show a variety of activities, such as antimicrobial, antitumour, anti-inflammatory, cytotoxic, antiviral, antimalarial, antibacterial and antifungal. Sesquiterpene lactones also have effects on the central nervous and cardiovascular systems and some metabolites also show allelopathic properties (Chadwick et al., 2013). Some of these properties could prove useful for the development of new agrochemicals.

Synthetic herbicides have been used intensively in an effort to achieve maximun productivity, but this has led to various environmental (Vieria et al., 2016) and resistance problems (Owen, 2016). Resistance is caused by the repetitive use of herbicides with the same mode of action and persistence in the soil. For these reasons, there is an urgent need to change the strategy applied in the research and development of herbicides. Therefore, an understanding of how plants interact with their environment to produce bioactive metabolites may offer potential to solve the problems caused by synthetic herbicides (Macias et al., 2007).

Allelopathy is the influence that a plant has on a target species, which can include plants, algae, bacteria or fungi, through the release into the environment of compounds (allelochemicals) that influence the growth and development of biological systems (Rice 1984; Zeng et al., 2008). Allelochemicals such as diterpenes or sesquiterpene lactones can be used as models in the development of herbicides of natural origin. Natural products represent an important alternative due to their huge structural diversity and their wide spectrum of biological activities.

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The advantages of these herbicides could be the absence of halogenated molecules, alternative modes of action, higher specific activity for weed management, the lower concentrations required for activity and the lower levels of environmental damage (Macías et al., 2008). For these reasons the discovery of new allelochemicals is an attractive alternative to current conventional herbicides used for weed control.

The first step in the development of these new herbicides should be the search for new models. However, plants produce a huge number of natural products with multiple activities and it is therefore difficult to identify all bioactive compounds of interest. As a consequence, it is necessary to use a methodical procedure that involves carrying out assays on compounds consistently in order to identify the components that are active. Specifically, the etiolated wheat coleoptile bioassay was used as an initial approach to evaluate the phytotoxicity of the compounds under investigation since it is a rapid test (24 h) that is sensitive to a wide range of bioactive substances (Cuttler et al., 2000), including plant growth regulators, herbicides (Cuttler, 1984), antimicrobials, mycotoxins and assorted pharmaceuticals (Jacyno and Cutler, 1993). Second, in order to manage the problems caused by weeds, their target plants must be selected for a standard phytotoxicity bioassay. Macias et al. (2000) proposed four standard target species as being representative of the most important taxonomic groups for both monocotyledons and dicotyledons. These species can be used as models for the most important weeds and they offer better properties (germination, predictable and reproducible behaviour, genetic homogeneity, sensitivity, etc.). Finally, a phytotoxicity assay using noxious weeds is necessary to assess the phytotoxicity of the compound in question. For this test, two important weed species were selected from around the world, namely barnyardgrass (*Echinochloa crus-galli* L.) and brachiaria [*Urochloa decumbens* (Stapf) R.D. Webster] belonging to the Poaceae family. Barnyardgrass is native to Asia and it is an invasive weed in rice plantations around the world (Talbert and Burgos, 2007). Barnyardgrass is the third most problematical weed worldwide as it causes losses of up to 70% in rice plantations (Mitich, 1991) and can also affect other cultures such as cotton, corn and potato (Holm et al., 1977). Barnyardgrass has also developed resistance to conventional synthetic herbicides (Talbert and Burgos, 2007). Brachiaria is native to Africa and it is also especially invasive in South America (Souza et al., 2006). These plants were introduced to Brazil to serve as pasture but they have spread throughout the country (Williams and Baruch, 2000). Brachiaria are very competitive to native plants, more tolerant to fire and they can markedly modify the environment in which they dominate (D'Antonio and Vitousek, 1992).

The aim of the work described here was to evaluate the bioactivity profiles of eight heliangolide sesquiterpene lactones (**1**–**8**) (Bautista et al., 2012a, 2014a; Calzada et al., 2009), one guaianolide sesquiterpene lactone (**9**) (Fronczek et al., 1984) and twenty two diterpenes (**10**–**31**) (Bautista et al., 2012b, 2013a,b, 2014b; Maldonado and Ortega 2000; Rodriguez-Hahn et al., 1990; Narukawa et al., 2006; Esquivel et al., 2005) isolated from species of the genera *Decachaeta*, *Salvia* and *Podachaenium* (Fig. 1). Our hypothesis is that these compounds could inhibit the development of invasive weeds such as barnyardgrass and brachiaria and they could be used in the development of natural herbicide models.

2. Results and discussion

2.1. Coleoptile bioassay results

The results are shown in Figs. 2 and 3. All of the sesquiterpene lactones assayed were active. Of these compounds, **1**, **5**, **6**, **7** and **9** showed the most consistent profiles, and showed higher phytotoxic activity than did Logran®, with levels of inhibition greater than 85% at the first three concentrations tested (10^{-3} M, $3 \cdot 10^{-4}$ M and 10^{-4} M). Compounds **6**, **7** and **9** also showed high activity levels at the fourth concentration tested ($3 \cdot 10^{-5}$ M), with values of 70, 55 and 70%, respectively. From these compounds, the molluscicidal (Fronczek et al., 1984) and antimycobacterial activity (Cantrell

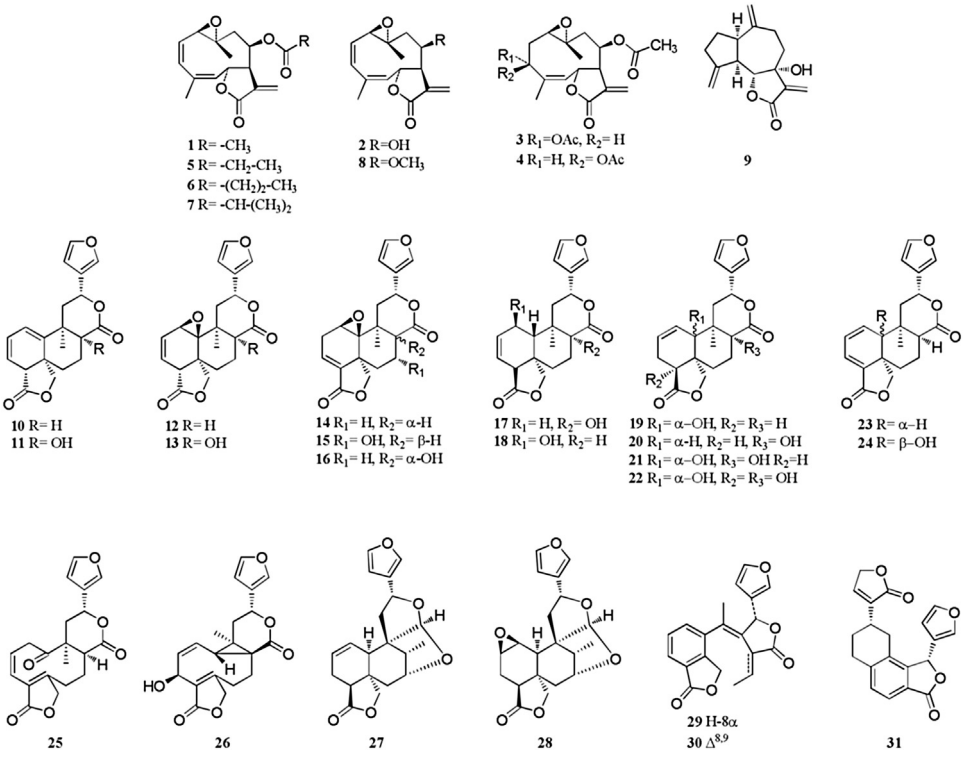

Fig. 1. Structures of compounds tested in the bioassay.

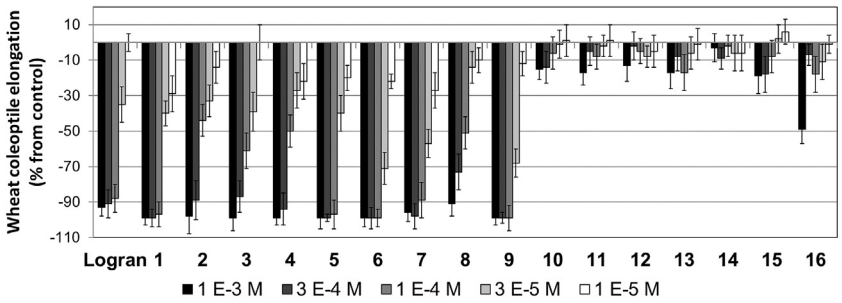

Fig. 2. Effects of compounds **1**–**16** and the herbicide Logran® on the elongation of etiolated wheat coleoptiles. Values are expressed as percentage difference from control.

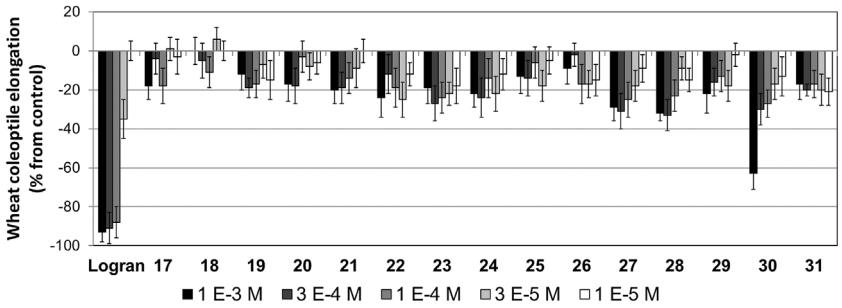

Fig. 3. Effects of compounds **17**–**31** and the herbicide Logran® on the elongation of etiolated wheat coleoptiles. Values are expressed as percentage difference from control.

et al., 1998) of guaianolide **9** was reported previously. The heliangolides **1**, **5**, **6** and **7** differ only in the ester chain at position 8, with **6** and **7** bearing a longer ester side chain, and their lipophilicity could improve the inhibitory effects at higher dilution. In contrast, compounds **2**, **3**, **4** and **8** lost their inhibitory activity more rapidly with dilution, with a value of less than 60% at 10^{-4} M. Firstly, compounds **2** and **8** differ from **1** in the absence of an ester on C-8, which bears a hydroxyl and a methoxyl group, respectively, so the presence of an ester group at C-8 seems to increase the activity. Secondly, compounds **3** and **4** do not have a double bond between C-2 and C-3 but they do contain an acetyl group at C-3.

As far as the diterpenes (**10**–**30**) were concerned, only compounds **16** and **30** showed relevant inhibitory effects at 10^{-3} M (50 and 65%, respectively).

The changes observed in the phytotoxicity with lipophilicity for the heliangolides appear to be consistent with Hansch's model (Hansch and Fujita, 1964). In an effort to assess the relevance of lipophilicity, a plot of Log P versus phytotoxicity is shown in Fig. 4. Similar correlations have previously been made for other sesquiterpene lactones that also have an ester chain in their structure (Macías et al., 2005). The authors employed the wheat coleoptile bioassay as a way to predict phytotoxicity and good correlations were found between phytotoxicity and LogP. The correlations identified from the results obtained for the screened heliangolides are presented here. The phytotoxicity [expressed as $\log(1/IC_{50})$] was correlated with cLogP and MLogP as shown in Fig. 4. A quadratic correlation between IC_{50} and LogP was observed for compounds **1**–**8**. IC_{50} decreased with LogP and this finding corroborates our hypothesis concerning the length of the side chain. The LogP values were lower than the Tice (2001) and Lipinski (1995) maximum values (4 and 5, respectively) and one would therefore expect a minimum IC_{50} on increasing the length of side chain and then an increase in this parameter. Furthermore, the ester groups in compounds **1**, **5**, **6** and **7** may be rapidly hydrolysed in plant systems to produce compound **2** and therefore a correlation between lipophilicity and activity for these compounds is likely due to the greater uptake of the higher LogP compounds followed by production of the same active compound. However,

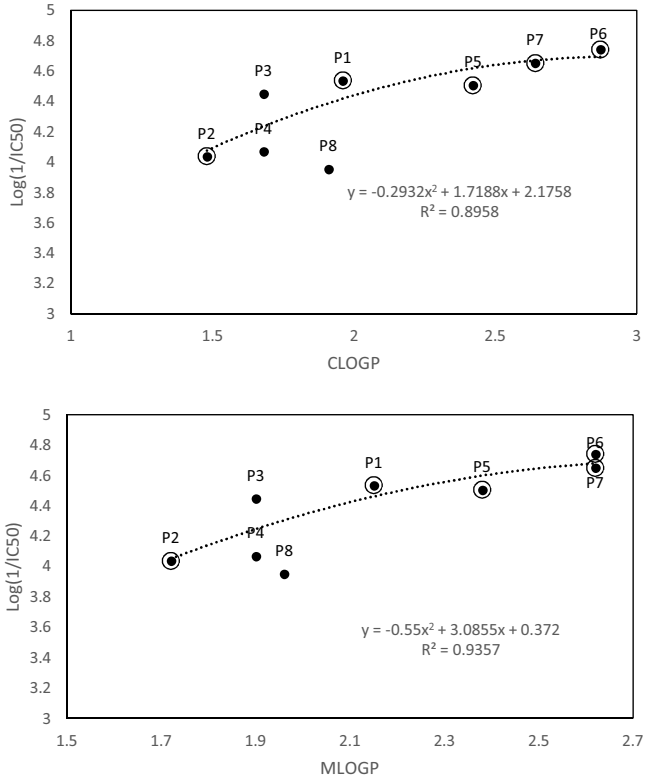

Fig. 4. Correlation of $\log(1/IC_{50})$ vs cLogP and MLogP obtained for heliangolides **1**–**8**. A quadratic correlation between IC_{50} and LogP was observed for P2 and its ester derivatives which are marked.

compound **8**, that cannot be easily hydrolyzed into **2**, is the least active heliangolide and the presence of the methoxyl group at C-8 appears to decrease the activity. On the other hand, compounds **3** and **4** do not have a double bond between C-2 and C-3 but they

contain an acetyl group at C-3. This double bond could be an important feature for the bioactivity of heliangolides. Finally, epimers **3** and **4** showed markedly different activities, so the stereochemistry at C-3 is important in the phytotoxicity of heliangolides.

2.2. Phytotoxicity bioassay

The most active compounds, namely the sesquiterpene lactones **1**–**9** and the diterpenes **16** and **30**, were selected for evaluation of the phytotoxicity on the standard target species (STS) *Lepidium sativum* L. (cress), *Lactuca sativa* L. (lettuce), *Solanum lycopersicon* L. (tomato) and *Allium cepa* L. (onion), and on two weed species, namely barnyardgrass (*Echinochloa crus-galli* L.) and brachiaria (*Urochloa decumbens* (Stapf) R.D. Webster).

The results of the bioassay are represented in Figs. 5–7 . It can be seen from the cluster analysis in Fig. 8 that on STS the most active compounds were sesquiterpene lactones, with **9** showing the highest activity and **1**, **2**, **5** and **6** showing high activity. The parameter that was affected the most was root length, whereas the least affected parameter was germination. These compounds were active on tomato, cress and onion roots at the first dilution tested, with inhibitory effects of around 90% on tomato and 80% on cress and onion. A hormesis effect was produced by **1** on onion, with stimulatory effects on growth close to 60% at 10^{-5} M. In addition, compounds **1**, **5**, **6** and **9** showed inhibitory effects at the second dilution tested (**1**, **5** and **6**, with values around 90% on tomato; **1**, **6** and **9** between 70 and 55% on cress; and **1** at 50% on onion).

Regarding the species affected, all sesquiterpene lactones showed inhibitory activity on tomato, which was the most sensitive plant, with values greater than 60% on root and greater than 40% on shoot at 10^{-3} M (on shoot, **1**, **5**, **6** and **9** were again the most active compounds – with activities greater than 50% at $3 \cdot 10^{-4}$ M). Lettuce was the species that was affected the least, with only the root affected by **1**, **2**, **3**, **4** and **9**. On cress and onion shoots the inhibitory effects of **1**, **2**, **6** and **9** were also relevant (**1**, **2** and **9** showed values greater than 70% on cress and **6** and **9** gave values greater than 60% on onion).

Regarding the diterpenes, the inhibition caused by **16** on tomato and onion roots was significant (50%), as were the stimulatory effects of **30** on lettuce root at the first three concentrations tested (50, 45 and 35% respectively). In summary, as in the coleoptile bioassay, lactones **1**, **2**, **4**, **5**, **6** and **9** were the most active compounds and they showed inhibitory effects against most STS

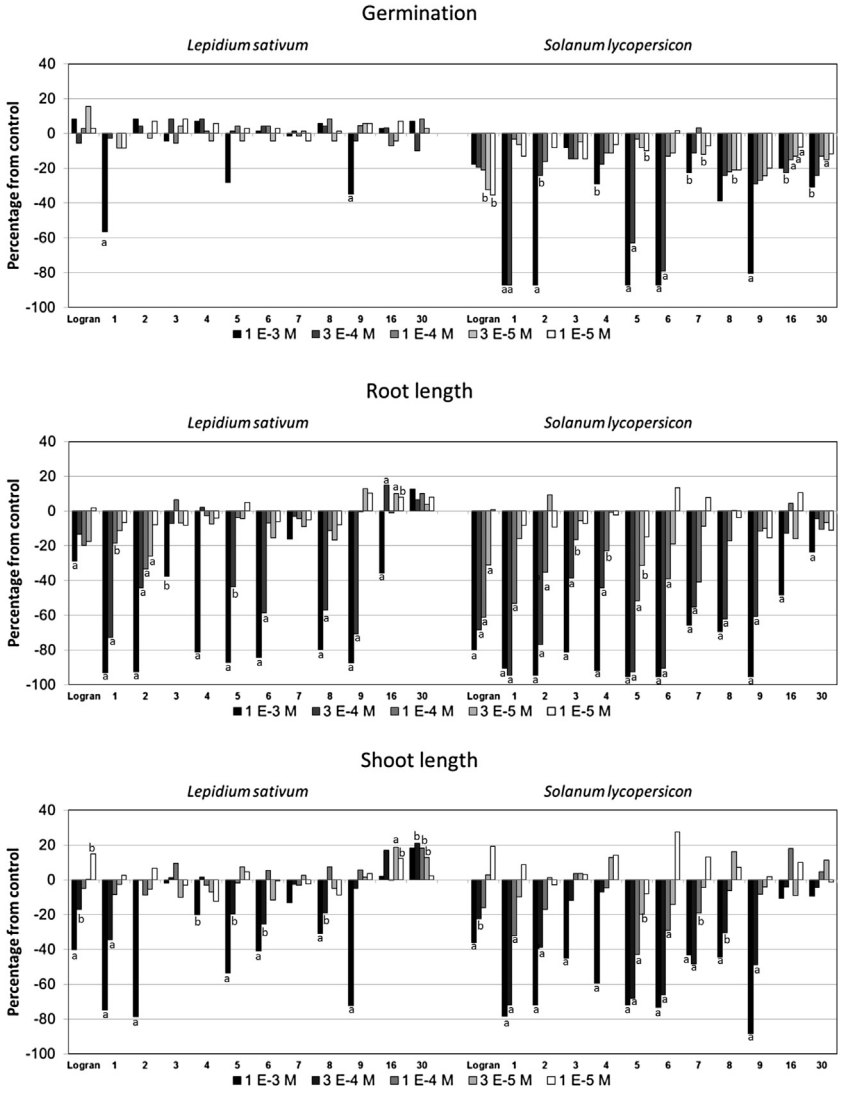

Fig. 5. Effects of compounds **1**–**9**, **16** and **30** on *Lepidium sativum* and *Solanum lycopersicon*. Values are expressed as percentage difference from control.

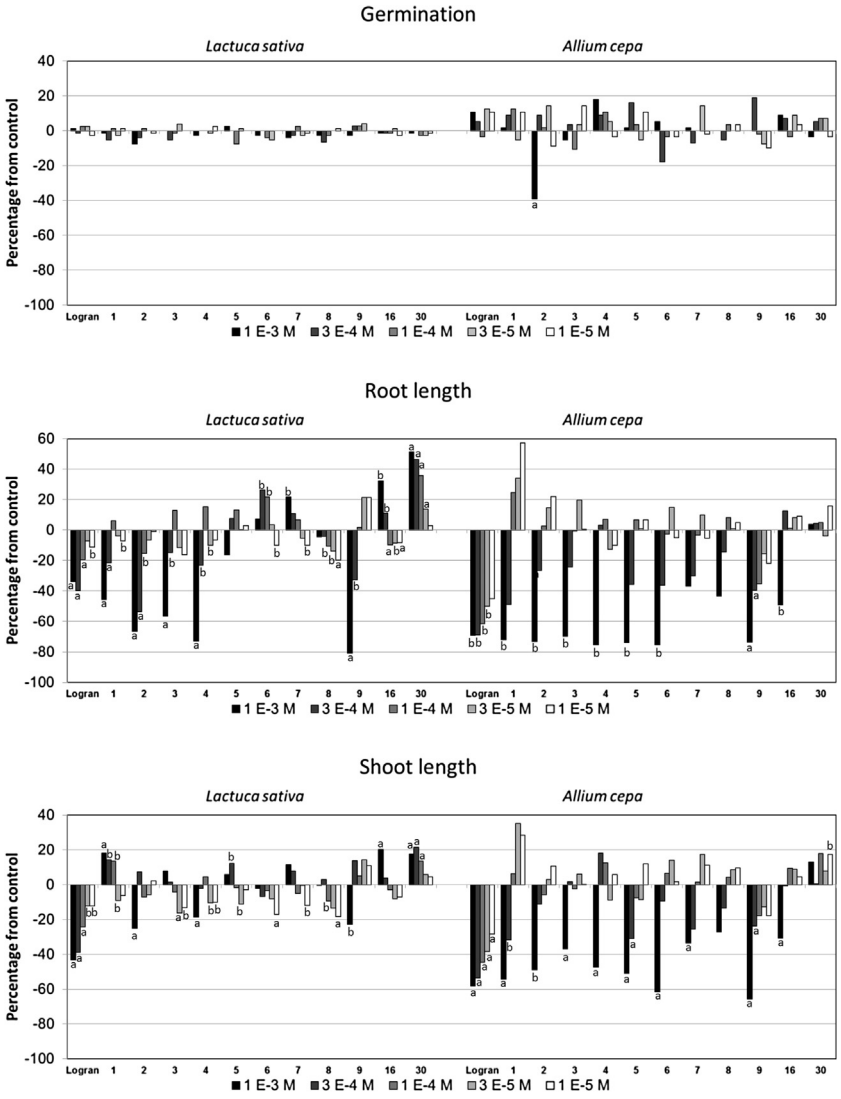

Fig. 6. Effects of compounds **1**–**9**, **16** and **30** on *Lactuca sativa* and *Allium cepa*. Values are expressed as percentage difference from control.

seeds and for most parameters tested. In addition, compounds **1** and **9** were active at the second dilution tested in certain cases and these showed the best activity profiles.

The cluster analysis for these compounds on weed seeds is represented in Fig. 9. The compounds evaluated can be ranked as follows: compound **9**, which is the most active example, those compounds with high activity (**1**, **2**, **4**, **5** and **6**) and those with low activity. Analysis of the results for weeds, in a similar way to STS, showed that brachiaria was the most sensitive weed. These compounds showed activity on all of the parameters tested. Germination was only affected on brachiaria. In this respect, compound **9** showed inhibitory effects at the two first concentrations tested (80 and 70%, respectively), compounds **1** and **2** showed 80% inhibition at 10^{-3} M, compounds **4**, **5** and **6** showed inhibitory activity greater than 70% at 10^{-3} M, and compound **5** was also active at $3 \cdot 10^{-4}$ M (70%). As far as the activity on shoot length is concerned, compounds **1** and **2** showed inhibitory effects at 10^{-3} M on brachiaria (around 80%). Compounds **4** and **9** were also active, with inhibitions of 50 and 45%, respectively. Only compound **9** showed inhibitory effects on barnyardgrass shoot (45% at 10^{-3} M). Regarding activity on root length, this was once again the parameter that was affected the most, with compounds **1**, **2**, **4**, **5**, **6** and **9** showing inhibitory effects greater than 80% at 10^{-3} M for both species. At $3 \cdot 10^{-4}$ M compounds **9** and **6** were also active on barnyardgrass (90 and 55%, respectively) and **1**, **2** and **9** were active on brachiaria (60, 50 and 70%, respectively). Compounds **7** and **8** also showed inhibitory activity at the first two concentrations tested on brachiaria (close to 70% at the first and greater than 50% at the second concentration). Epimers **3** and **4** showed markedly different activities on the root length of weeds (barnyardgrass and brachiaria) and cress, which indicates that the stereochemistry of the acetyl moiety at C-3 is important for the phytotoxicity of heliangolides. It is important note the hormesis effect shown on the root of barnyardgrass by **4** and **8**, with stimulatory effects close to 40% at the final dilutions.

In summary, the results presented above allow several conclusions to be drawn:

1. All sesquiterpene lactones were active on coleoptiles and reduced length of coleoptiles by at least 92%. As far as heliangolides are concerned, lipophilicity is a key factor for activity and higher activities were observed as logP increased. LogP values were lower than the Tice and Lipinski maximum values (4 and 5, respectively) so one would expect a minimum

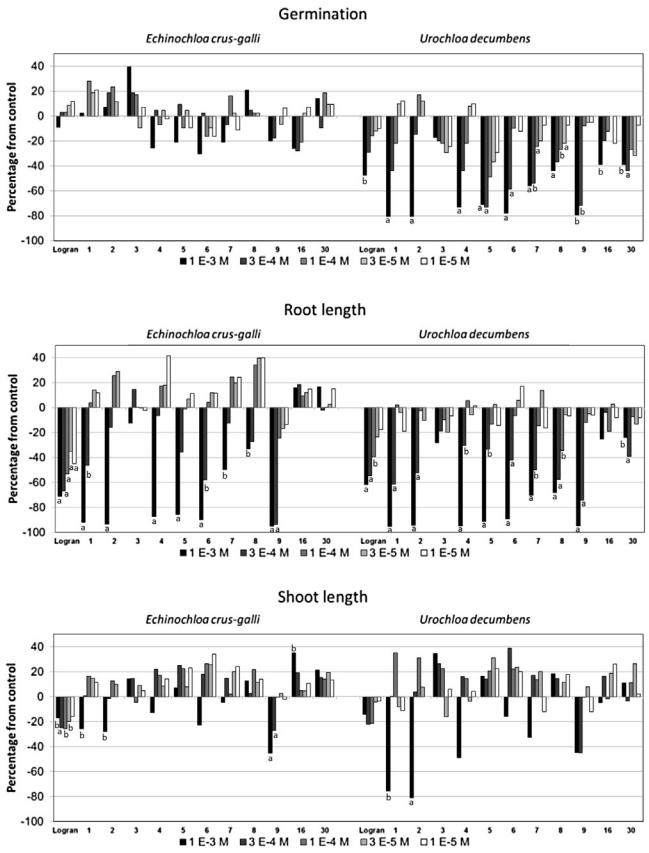

Fig. 7. Effects of compounds **1**–**9**, **16** and **30** on weed growth. Values are expressed as percentage difference from control.

IC_{50} value on increasing the length of side chain and then an increase.

2. Diterpenes were generally inactive. Only **16** and **30** showed some inhibitory effects. It is important to note the stimulatory effect of **30** on lettuce roots.
3. Compounds **1**, **2**, **4**, **5**, **6** and **9** showed inhibitory effects on STS and weeds and **1**, **6** and **9** showed the best profiles, with activity observed at the second dilution tested.
4. The promising results obtained clearly show that **1**, **2**, **4**, **5**, **6** and **9** warrant further investigation to evaluate their potential for the development of novel herbicides of natural origin.

The route to more integrated and environmentally friendly agriculture requires models for the development of new herbicides based on natural strategies. The results described here show that natural products, and particularly sesquiterpene lactones, are valuable resources and these remain relatively unexplored.

3. Experimental

3.1. Isolation of compounds

Sesquiterpene lactones **1**–**4** were isolated from the CH_2Cl_2-soluble extract of the leaves from *Decachaeta incompta* (Bautista et al., 2012b). Compounds **5**–**8** are semi-synthetic derivatives of incomptine B (**2**) and they were obtained according to a previously described procedure (Bautista et al., 2014b). The guaianolide **9** was isolated in large quantities from a $CHCl_3$-soluble extract of the leaves from *Podachaenium eminens* as described by Fronczek and co-workers (Fronczek et al., 1984).

Diterpenes **10**–**16** were isolated from the aerial parts of *Salvia herbacea* (Bautista et al., 2012b). The diterpenes **17**–**22** were obtained from an acetone extract of the leaves from *S. shannoni* (Bautista et al., 2013a). Compounds **23**–**26** were isolated from the leaves and flowers of *S. microphylla* (Bautista et al., 2013b, 2014b).

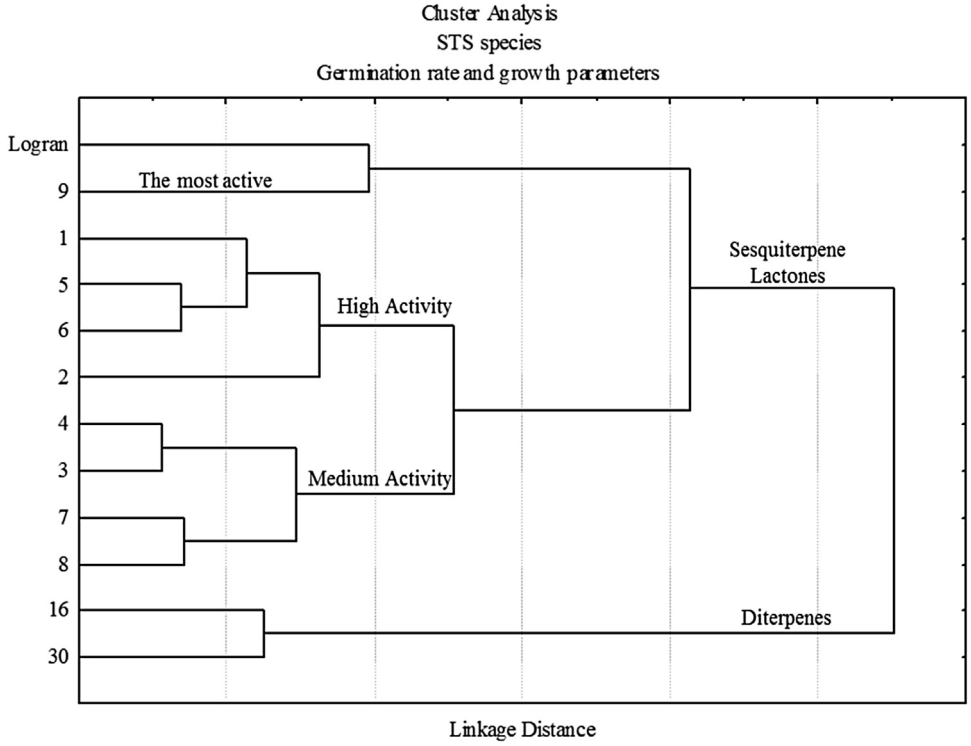

Fig. 8. Cluster analysis for the sesquiterpene lactones **1**–**9** and diterpenes **16** and **30** carried out based on germination and growth effects for STS species.

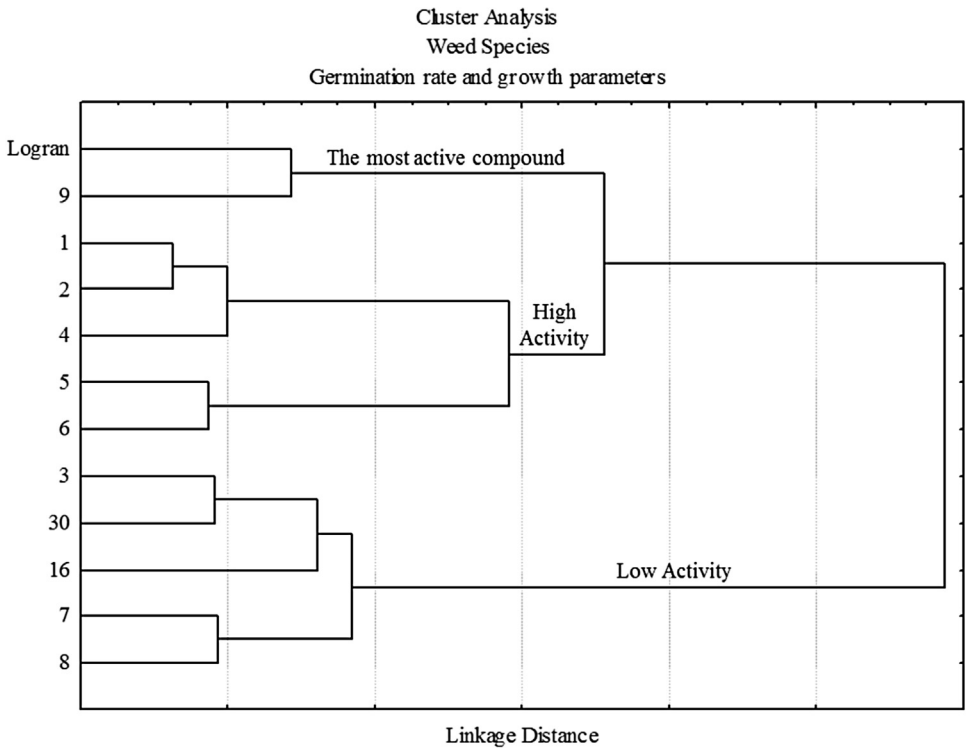

Fig. 9. Cluster analysis for the sesquiterpene lactones **1–9** and diterpenes **16** and **30** carried out based on germination and growth effects for weed species.

The clerodanes **27** and **28** were isolated from an acetone extract of the aerial parts of *S. polystachya* according to a literature procedure (Maldonado and Ortega, 2000). Diterpene **31** was isolated from the aerial parts of *S. tiliaefolia* (Rodriguez-Hahn et al., 1990).

*Isolation of compounds **29** and **30**. Plant material:* The aerial parts of *Salvia gesneriflora* were collected close to km 64 of the México-Cuernacava highway in November 2011 and the samples were identified by M. Sci. María del Rosario García Peña. A voucher specimen (MEXU-1320390) was deposited at The National Herbarium, Instituto de Biología, Universidad Nacional Autónoma de México.

3.2. Extraction and isolation

The dried and powdered plant material (2.1 kg) was extracted by percolation with acetone (12 L) to give a gummy residue (96.3 g). The acetone-soluble extract was dissolved in 0.5 L of a mixture MeOH/H_2O (4:1) and partitioned with Hexane (0.4 L × 10). The hydroalcoholic fraction was concentrated to one fifth of its original volume and partitioned again with EtOAc (0.5 L × 3). The EtOAc fraction (34 g) was submitted to vacuum column chromatography (VCC) eluting with hexane/EtOAc 4:1 (fraction A), hexane/EtOAc 3:1 (fraction B), hexane/EtOAc 7:3 (fraction C), and hexane/EtOAc 3:2 (fraction D). Fraction A (2.86 g) was submitted to successive vacuum column chromatography (VCC) eluting with $CHCl_3$/EtOAc 97:3, hexane/EtOAc 3:1, and hexane/EtOAc 7:3 to obtain, after crystallization from EtOAc/hexane, 173 mg of compound **29**. Fraction B (3.42 g) was subjected to successive VCC eluting with hexane/$CHCl_3$/MeOH 60:40:1 and hexane/EtOAc/MeOH 80:20:1 to obtain, after crystallization from EtOAc/hexane, 87.6 mg of compound **30**. Compounds **29** and **30** were identified by comparison of their ^{1}H and ^{13}C NMR spectra with those described for salvifulgenolide (Narukawa et al., 2006) and isosalvixalapadiene (Esquivel et al., 2005), respectively.

3.3. Coleoptile bioassay

Wheat seeds (*Triticum aestivum* L. cv. Catervo) were sown in 15 cm diameter Petri dishes moistened with water and grown in the dark at 25 ± 1 °C for 4 days (Hancock et al., 1964). The roots and caryopses were separated from the shoots. The latter were placed in a Van der Weij guillotine and the apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for bioassays. All manipulations were performed under a green safelight (Nitsch and Nitsch, 1956). Pure compounds were predissolved in dimethyl sulfoxide (DMSO) (0.1%) and diluted in phosphate-citrate buffer containing 2% sucrose (Nitsch and Nitsch, 1956) at pH 5.6 to the final bioassay concentrations (10^{-3}, $3 \cdot 10^{-4}$, 10^{-4}, $3 \cdot 10^{-5}$ and 10^{-5} M).

Parallel controls were also run. The commercial herbicide Logran®, whose original formulation is a combination of N^2-*tert*-butyl-N^4-ethyl-6-(methylsulfanyl)-1,3,5-triazine-2,4-diamine (terbutryn, 59.4%) and 2-(2-chloroethoxy)-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl]benzene-1-sulfonamide (triasulfuron, 0.6%), was used as an internal reference according to a comparison study reported previously (Macias et al., 2000). This reference was used at the same concentrations and under the same conditions as reported previously. Control samples (buffered aqueous solutions with DMSO and without any test compound) were used for all of the plant species assayed.

Five coleoptiles and 2 mL of solution were placed in each test tube (three tubes per dilution) and the tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 25 °C in the dark. The coleoptiles were measured by digitalization of their images. Data were statistically analysed using Welch's test (Martín Andrés and Luna Del Castillo, 1990). Data are presented as percentage differences from control. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition.

3.4. Phytotoxicity bioassays

The selection of target plants was based on an optimization process carried out in our search for a standard phytotoxicity bioassay (Macias et al., 2000). Several Standard Target Species (STS) were proposed, including the monocotyledon onion (*Allium cepa* L.) and the dicotyledons tomato (*Solanum lycopersicon* L.), cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.), which were all assayed in this study. In addition, two weed species were added as target plants in this bioassay: barnyardgrass (*Echinochloa crus-galli* L.) (the most important weed in rice plantations around the world) and brachiaria (*Urochloa decumbens* (Stapf) R.D. Webster) (the most important invasive weed in South America).

Bioassays were conducted using Petri dishes (50 mm diameter) with one sheet of Whatman No.1 filter paper as a support. Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-[*N*-morpholino]ethanesulfonic acid (MES) and 1 M NaOH (pH 6.0). Compounds to be assayed were dissolved in DMSO and these solutions were diluted with buffer (5 μL DMSO solution/mL buffer) so that test concentrations (10^{-3}, $3 \cdot 10^{-4}$, 10^{-4}, $3 \cdot 10^{-5}$ and 10^{-5} M) were achieved. Parallel controls were also run as described previously for coleoptile bioassays.

Four replicates were used for tomato, cress, onion, lettuce, barnyardgrass and brachiaria, and each replicate contained 20 seeds. Treatment, control or internal reference solution (1 mL) was added to each Petri dish. After adding the seeds and aqueous solutions, the Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were further incubated at 25 °C in a Memmert ICE 700 controlled environment growth chamber. The photoperiod was 24 h of dark for onion, tomato, cress and lettuce, and 16/8 h light/dark for barnyardgrass and brachiaria. Bioassays took 4 days for cress, 5 days for tomato, 6 days for lettuce, 7 days for onion and 8 days for barnyardgrass and brachiaria. After the growth period, plants were frozen at −10 °C for 24 h in order to avoid subsequent growth during the measurement process.

Evaluated parameters (germination rate, root length and shoot length) were recorded using a Fitomed© system (Macias et al., 2000), which allowed automatic data acquisition and statistical analysis using its associated software. Data were analysed statistically using Welch's test (Martín Andrés and Luna Del Castillo, 1990), with significance fixed at 0.01 and 0.05. Results are presented as percentage differences from the control. Zero represents control, positive values represent stimulation, and negative values represent inhibition. Statistical significance is expressed by means of letters, where 'a' denotes significantly different from control with 0.01 confidence and 'b' indicates different from control with a confidence from 0.01 to 0.05. The absence of a letter indicates no significant difference from control values.

Once the germination and growth data had been acquired, cluster analysis was used to group compounds with similar phytotoxicity behaviours and to associate the clusters with the molecular structure. Complete linkage was used as an amalgamation rule and the distance measurement was based on squared Euclidean distances, given by the equation

$$d(x,y) = \sum_i (x_i - y_i)^2$$

where d(*x*,*y*) is the squared Euclidean distance (*i*-dimensional), *i* represents the number of variables, and *x* and *y* are the observed values. The cluster was obtained using Statistica v.7.0 software (Statistica 7.0, Tulsa, OK,USA.). Germination rate, shoot length and root length effects for STS were included in the analysis in order to acquire an overall view of the phytotoxicity and its relationship with chemical structure

Table 1
Lipophilicity and IC_{50} values for compounds tested in the coleoptile bioassay.

Compound	IC_{50} (M)[R^2]	cLogP	MLogP
1	$2.9 \cdot 10^{-5}$ [0.96]	1.96	2.15
2	$9.2 \cdot 10^{-5}$ [0.97]	1.48	1.72
3	$3.5 \cdot 10^{-5}$ [0.99]	1.68	1.90
4	$8.5 \cdot 10^{-5}$ [0.97]	1.68	1.90
5	$3.1 \cdot 10^{-5}$ [0.96]	2.42	2.38
6	$1.8 \cdot 10^{-5}$ [0.96]	2.87	2.62
7	$2.2 \cdot 10^{-5}$ [0.99]	2.64	2.62
8	$1.1 \cdot 10^{-4}$ [0.99]	1.91	1.96
9	$2.1 \cdot 10^{-5}$ [0.94]	1.80	2.52
16	$1.4 \cdot 10^{-3}$ [0.96]	0.03	1.31
30	$7.3 \cdot 10^{-4}$ [0.97]	2.28	2.80

3.5. Calculation of half maximum inhibitory concentration (IC_{50}) and lipophilicity (logP)

The IC_{50} values (Table 1) were calculated by fitting the data to a dose-response sigmoidal curve with variable slope using the PRISMA 5 package (PRISMA 5.0, San Diego, CA, USA.) in a similar way to the values used in herbicide studies (Schabenberger et al., 1999). Calculated logarithm of Partition coefficient (cLogP) values were obtained using the Osiris property explorer (Sander, 2001) and Moriguchi logarithm of Partition coefficient (MLogP) values were obtained using the software ALOGPS 2.0 (VCCLAB, 2005) (Table 1).

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