

# Evaluation of the phytotoxic and antifungal activity of C<sub>17</sub>-sesquiterpenoids as potential biopesticides

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## Abstract

**BACKGROUND:** Natural products are a promising source for the development of new pesticides with alternative mechanisms of action. In this study, we evaluated the phytotoxic and antifungal activity of a novel family of natural C<sub>17</sub>-sesquiterpenoids and performed a study of the effect caused by the elimination of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system and its importance to their biological activity.

**RESULTS:** Many tested compounds exhibited a strong phytotoxic activity. Lappalone and pertyolide B were the most potent molecules from the tested group. Lappalone displayed a strong inhibition profile against selected weed species, reaching a half-maximal inhibitory concentration (IC<sub>50</sub>) value of 5.0  $\mu$ M against *Echinochloa crus-galli* L. shoot and 5.7  $\mu$ M against the germination rate of *Amaranthus viridis* L., as well as a good stimulation of the germination of *Phelipanche ramosa* L. Pertyolide B demonstrated excellent inhibition against *Amaranthus viridis* L. (IC<sub>50</sub>: 56.7, 70.3 and 24.0  $\mu$ M against the root and shoot growth, and germination rate, respectively) and *Allium cepa* L. (representative of the Liliaceae family, with IC<sub>50</sub> values of 25.3 and 64.4  $\mu$ M against root and shoot growth). Regarding the antifungal activity, pertyolide B presented significant activity against *Colletotrichum fragariae* and *Fusarium oxysporum* with a minimum inhibitory concentration of 6.6  $\mu$ g  $\mu$ L<sup>-1</sup>.

**CONCLUSION:** The bioassays revealed that frequently the presence of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system is not essential for the bioactivities of sesquiterpene lactones, and suggest that C<sub>17</sub>-sesquiterpenoids may function through a different mechanism of action not related to the widely assumed Michael addition.

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**Keywords:** C<sub>17</sub>-sesquiterpenoids; natural products; pest control; pesticide

## 1 INTRODUCTION

Pests are responsible for substantial losses in global crop production and have become a big concern for the agriculture industry. Since their advent in the 20th Century, synthetic herbicides have been the main tool to fight weeds to improve crop production. However, the excessive dependence on them and their abuse, especially with the appearance of glyphosate, led to the neglect of research on pesticides with novel mechanisms of action.<sup>1</sup> Worldwide, >200 active ingredients have been registered for herbicide use, but only 29 modes of action (MoA) have been reported.<sup>2</sup> As a consequence, pests have developed resistance to the exploited agrochemicals and became harder to control, making the research for new pesticides with alternative MoA a necessity.

Natural products are a promising resource in the development of alternatives to traditional synthetic agrochemicals<sup>3</sup> for several reasons. First, they are synthesized by living organisms as a result of the evolution of their response to biotic or abiotic stimuli.

Secondly, they also are generally produced through complex synthetic pathways leading to molecules not easily accessible with conventional synthetic procedures in the laboratory, giving access to structures with different target sites than those already exploited by modern herbicides.<sup>4,5</sup>

Sesquiterpene lactones (SLs) are one of the largest family of natural products and a broad range of bioactivities already have been

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reported. SLs are isolated mainly from the aerial parts of plants of the Compositae family<sup>6</sup> although they also can be found in other plant families, such as Umbelliferae, Lauraceae and Magnoliaceae.<sup>7</sup> A biosynthesis of SLs with high phytotoxic and antifungal activity,<sup>8–10</sup> by many weed species generated interest in their possible application for pest management.<sup>11,12</sup>

Recent isolation studies have led to the discovery of a novel family of SLs commonly named C<sub>17</sub>-sesquiterpenoids.<sup>13–16</sup> These compounds possess an unusual carbonated skeleton of 17 units and have eliminated the  $\alpha$ -methylene- $\gamma$ -butyrolactone moiety to add a carbonated chain in its place. The lack of this moiety is of great interest, as frequently it is associated with the bioactivity of SLs through a Michael addition with a nucleophilic substrate.<sup>17,18</sup>

Up-to-date studies on the biological activities of C<sub>17</sub>-sesquiterpenoids are very limited. Only a few medical activities have been reported,<sup>13–16</sup> however, no information on their application in agriculture can be found. In our previous reports,<sup>19,20</sup> we noticed that some of the C<sub>17</sub>-sesquiterpenoids, as well as some intermediates synthesized without the  $\alpha$ -methylene- $\gamma$ -butyrolactone moiety presented good phytotoxic activity, in some cases even better than the parent SL. This fact attracted our attention that these molecules may hide a key to a possible novel MoA. Thereby, in the present work, we have carried out an in-depth evaluation of the phytotoxic and antifungal activity of this kind of molecule to further analyze their potential in the field of pest management.

In order to achieve this, we have assessed the phytotoxic and antifungal activity of four naturally occurring C<sub>17</sub>-sesquiterpenoids [lapalone (**11**) and pertyolides A (**3**), B (**6**) and C (**14**)], as well as three natural SLs [dehydrocostuslactone (**7**), isovalantolactone (**1**) and alantolactone (**4**)]. These molecules were tested along with some of their synthetic intermediates (**2**, **5**, **8–13**) to study the effect induced by the elimination of the  $\alpha$ -methylene- $\gamma$ -butyrolactone group on their bioactivity (Fig. 1). The chemical compounds were evaluated in a variety of bioassays as pre-emergence and postemergence herbicides against well-known weed species, as well as their potential in the control of parasitic weeds. In addition, we also determined the antifungal activity of the compounds against common phytopathogenic fungi species.

In a preliminary assay, the pre-emergence potential of the chemicals was evaluated against *Lactuca sativa* L. (cv Iceberg A) as dicotyledonous species and *Agrostis stolonifera* L. as monocotyledonous species. The phytotoxic activity was further studied in a postemergence assay against *Lemna paucicostata* L., a monocotyledonous species widely used to evaluate the toxicology of xenobiotic compounds to aquatic organisms.<sup>21,22</sup>

The compounds also were evaluated against four seeds: cress (*Lepidium sativum* L.), onion (*Allium cepa* L.), lettuce [*L. sativa* L. (cv Romana)] and tomato (*Solanum lycopersicum* L.) in the standard target species (STS) assay. These seeds were selected by our research group as models of four of the most common weed families (Brassicaceae, Liliaceae, Asteraceae and Solanaceae respectively).<sup>23</sup> Furthermore, the compounds also were assayed against four target weed seeds (*Lolium perenne* L., *Echinochloa crus-galli* L., *Amaranthus viridis* L. and *Megathyrus maximus* Jacq.).

Parasitic plants are a special type of weed. They depend on a host plant to acquire nutrients and subsists.<sup>24</sup> To ensure their survival they remain dormant in the soil until they recognize a chemical signal released by their host plant to germinate. Suicidal germination or 'honey pot' is a technique used for parasitic plant management. In this strategy, a germination inducer of the parasitic weed is administered on the soil in the absence of a host; when the parasitic seeds detect this chemical they germinate but because there is no host to parasitize they will die from starvation.<sup>25</sup> In this assay we study the potential of the compounds to stimulate the germination of three parasitic plants (*Orobancha cumana* Wallr., *Orobancha crenata* F. and *Phelipanche ramosa* L.) to examine their potential as pre-emergence parasitic weed control.

The antifungal activity of the compounds also was assayed against three phytopathogenic fungi: *Colletotrichum fragariae* Brooks., *Fusarium oxysporum* Schlechtend.: Fr. and *Botrytis cinerea* Pers.: F.

## 2 MATERIALS AND METHODS

### 2.1 Isolation and synthesis of the tested molecules

Isoalantolactone (**1**) and alantolactone (**4**) were isolated by column chromatography from *Inula helenium* roots and

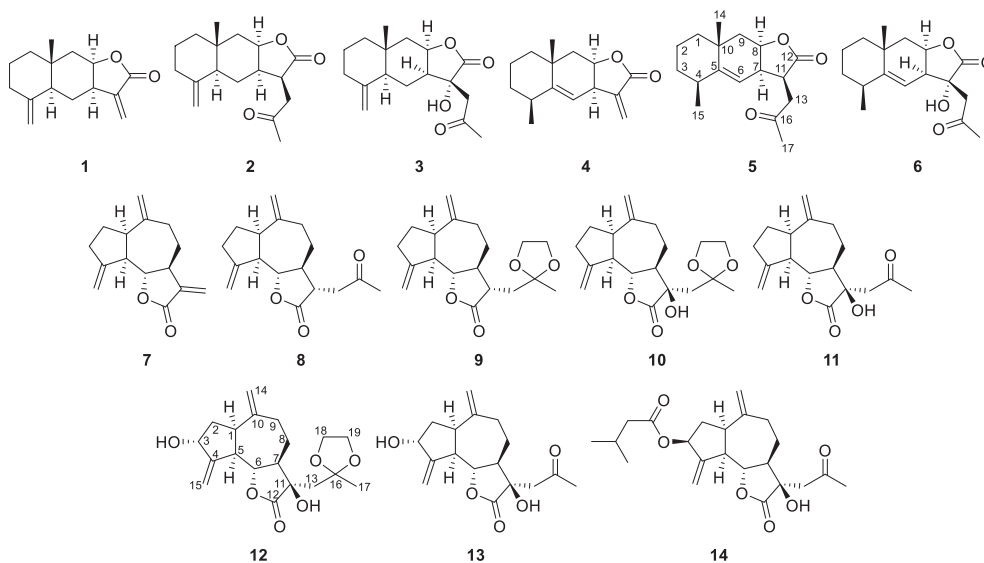


Figure 1. Chemical structures of the assayed compounds.

dehydrocostuslactone (**7**) was isolated by the same means from *Saussurea lappa* root extract. A detailed description of the isolation procedures and the synthesis of all the molecules tested (**2**, **3**, **5**, **6** and **8–14**) was described in our previous works.<sup>19,20</sup>

## 2.2 Seeds and fungi origin

*S. lycopersicum*, *L. sativa* (cv Romana), *A. cepa* and *L. sativum* seeds were generously provided by FITÓ. *E. crus-galli* and *L. perenne* seeds were purchased from Herbiseed (Reading, UK). *A. viridis* and *M. maximus* seeds were purchased from Agro Cosmos (Engenheiro Coelho, Brazil). *O. cumana* seeds were provided by Leonardo Velasco from the Institute for Sustainable Agriculture of CSIC (Córdoba, Spain), and *O. crenata* and *P. ramosa* seeds were generously provided by Professor Maurizio Vurro from Instituto di Scienze delle Produzioni Alimentari (Bari, Italy). *L. sativa* (cv Iceberg A) seeds were purchased from Burpee Seeds (Warminster, PA, USA) and *A. stolonifera* (cv Penncross) were supplied by Turf Seed Inc. (Hubbard, OR, USA). The *L. pausicostata* plants used were from a colony provided by Hiroshi Matsumoto of the University of Tsukuba (Japan) which has been maintained by the NPURU and used since the 1980s. The phytopathogenic fungi species were isolated from natural sources in the past and they have been maintained and used for bioassays as needed. *C. fragariae* was isolated from strawberry (*Fragaria* × *ananassa* Duchesne), *F. oxysporum* was isolated from orchid (*Cynoches* sp.) and *B. cinerea* was isolated from grape (*Vitis vinifera* L.).<sup>26</sup> The fungi species are kept stored at  $-80^{\circ}\text{C}$  in a 9:1 RPMI buffer-glycerol mixture with morpholinepropanesulfonic acid (MOPS) buffer at pH 7 until their use.

## 2.3 Preliminary assay for phytotoxic activity

A preliminary study was done on *L. sativa* and *A. stolonifera* following the procedure described in the literature.<sup>27</sup> The compounds to study were pre-dissolved in acetone and further diluted with distilled water to obtain a mixture with a fixed 10% rate of acetone of the concentrations to study (10.0 mM, 3.3 mM, 1.0 mM, 330.0  $\mu\text{M}$ , 100.0  $\mu\text{M}$ , 33.0  $\mu\text{M}$  and 10.0  $\mu\text{M}$ ). After the incubation period, the germinated seeds were digitalized using a LemnaTec Scanalyzer PL system (LemnaTec GmbH, Aachen, Germany) and analyzed. The commercial herbicide acifluorfen was used as positive control. In the case of *L. sativa*, the results were presented as percentage of root growth from the negative control with solvent, positive values mean stimulation, and negative values inhibition of the growth of the root. In the case of *A. stolonifera*, the results were presented as a percentage of germination from the negative control with solvent. The half-maximal inhibitory concentration (IC<sub>50</sub>) values were determined 7 days post-treatment with R software (v3.6.3) with the DRC package used in analysis of dose–response curves with a four-parameter logistic model.

## 2.4 Phytotoxic assay on *Lemna pausicostata*

The phytotoxic activity against *L. pausicostata* was evaluated following the procedure explained in the literature.<sup>27,28</sup> The compounds to study were pre-dissolved in acetone and further diluted with Hoagland media to obtain a mixture with a fixed 1% rate of acetone of the concentrations to study (1.0 mM, 330.0  $\mu\text{M}$ , 100.0  $\mu\text{M}$ , 33.0  $\mu\text{M}$ , 10.0  $\mu\text{M}$ , 3.3  $\mu\text{M}$ , 1.0  $\mu\text{M}$  and 0.33  $\mu\text{M}$ ). Each treatment was replicated three times. The commercial herbicide propa-nil was used as positive control. The frond area was measured using an image analysis system of the phenotyping instrument LabScanalyzer (LemnaTec). The half-maximal inhibitory concentration (IC<sub>50</sub>) values were determined 7 days post-treatment using R

(v3.6.3) with the DRC package used in analysis of dose–response curves with a four-parameter logistic model.

## 2.5 Phytotoxic assay on standard target species (STS) and target weeds

The evaluation of the compounds against the STS (*L. sativum*, *A. cepa*, *L. sativa* and *S. lycopersicum*) and the selected weeds (*A. viridis*, *E. crus-galli*, *L. perenne* and *M. maximus*) and the statistical analysis of the results were done following the procedure described in the literature.<sup>19</sup> The seeds were incubated for 4 days for *L. sativum*, 5 days for *S. lycopersicum*, 6 days for *L. sativa*, *A. viridis*, *E. crus-galli*, *L. perenne* and *M. maximus*, and 7 days for *A. cepa* before their measurement. Logran® was used as positive control. Three parameters were evaluated in these assays: the germination rate of the seeds and the elongation of the root and the shoot, all of them compared to the negative control. The data obtained were fitted to a dose–response curve [log(inhibitor) versus response] to determine the IC<sub>50</sub> values using PRISM v5.00 (GraphPad, San Diego, CA, USA).<sup>29</sup>

## 2.6 Broomrape seed germination assay

The bioassay against parasitic weed species (*O. crenata*, *O. cumana* and *P. ramosa*) was carried out following the procedure reported previously by our research group.<sup>30</sup> The natural strigolactone GR24 was used as positive control. The parameter to study in this assay was the germination rate of the seeds when exposed to different concentrations of the tested compounds. Data were subjected to ANOVA using PRISM v5.00 (GraphPad). The evaluation of the significance of mean differences between treatments and the negative control was made by two-sided Dunnett's test. The null hypothesis was rejected at the level of 0.05.

## 2.7 Antifungal assay

The antifungal activity of compounds **1–8** and **11** was evaluated against three species of filamentous fungi (*C. fragariae*, *F. oxysporum* and *B. cinerea*) following the thin layer chromatography (TLC) bioautography procedure described in the literature.<sup>31,32</sup> The effectiveness of the chemicals was measured by analyzing the diameter and the aspect (clear or diffuse) of the inhibition zones observed during the growth of the species after being exposed to different quantities of the molecules. In a primary assay, 100 and 20  $\mu\text{g}$  of the compounds were spotted by triplicate on the TLC plates (Uniplate™ Silica Gel GHLF Scored 10 × 20 cm, 250  $\mu\text{m}$ ). In the secondary assay, 100.00  $\mu\text{g}$ , 33.33  $\mu\text{g}$ , 10.00  $\mu\text{g}$ , 3.33  $\mu\text{g}$ , 1.00  $\mu\text{g}$  and 0.33  $\mu\text{g}$  of the compounds were spotted by triplicate on the TLC plates. The commercial fungicides benomyl, azoxystrobin, fludioxonil, chlorothalonil, dodine, thiram and prochloraz were used as positive controls.

# 3 RESULTS AND DISCUSSION

## 3.1 Preliminary assay for phytotoxic activity

Out of all tested molecules, lappalone (**11**) and alantolactone (**4**) revealed the best inhibition profiles (Fig. 2). The eudesmanolides-type molecules isovalantolactone (**1**) and alantolactone (**4**) presented strong inhibition activity at concentrations >1 mM and 330  $\mu\text{M}$ , respectively, with a huge drop in their activity at the lower concentrations. The elimination of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system diminished the phytotoxic activity, both compounds **2** and **5**, as well as pertyolides A (**3**) and B (**6**), exhibited strong stimulation of the root growth at concentrations >1 mM with a peak of strong inhibition for

**5** at 10 mM. The *L. sativa* seedlings responded in diverse manner to guaianolide-type compound treatment, dehydrocostuslactone (**7**) only presented moderate inhibition at the highest concentration but the elimination of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system caused a raise of the phytotoxic activity, especially, for lappalone (**11**) which presented a strong inhibition (50–70%) for concentration levels from 10 mM to 10  $\mu$ M. It is interesting to remark that in the case of compounds **5** and **8** we could observe cases of hormesis;<sup>33</sup> this occurs when high doses of a chemical compound produce an inhibition response in an organism but lower doses generate a stimulation response.

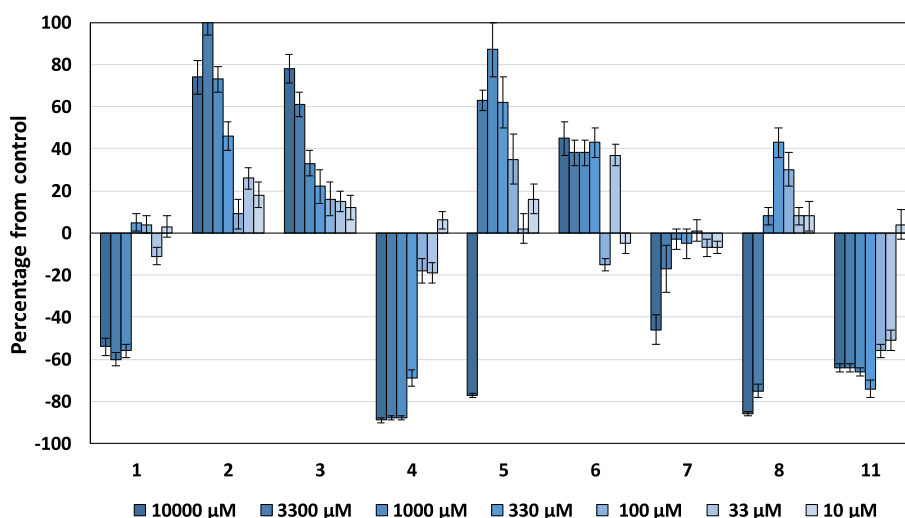
In the case of *A. stolonifera*, no significant difference was observed in the length of the shoots when the seeds germinated so the parameter of study for this species was the germination rate of the seeds after exposure to different concentrations of the test compounds compared to the negative control (Fig. 3; Table 1).

All of the compounds expressed strong inhibition of the germination of *A. stolonifera* except for pertyolide A (**3**) which only presented strong inhibition at 10 mM with a big drop of the activity afterward and a low stimulation at the lowest concentrations tested. There was not a clear tendency between

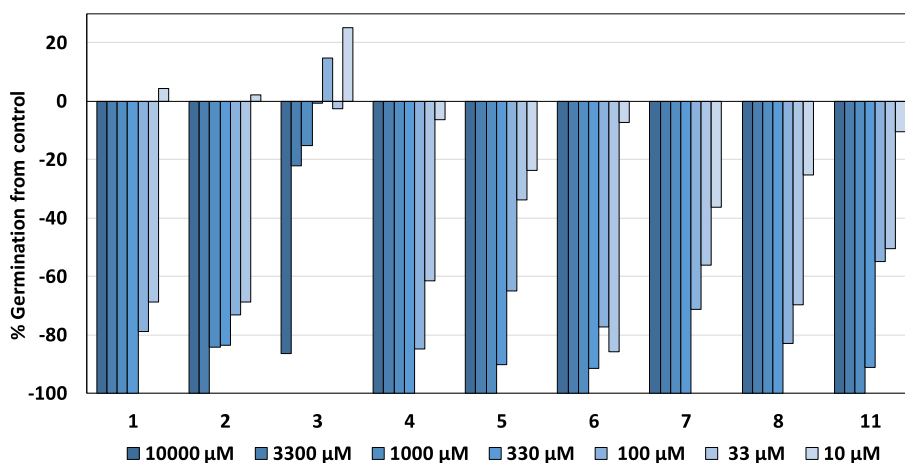
the structural modifications carried out and the activities observed. In the case of dehydrocostuslactone (**7**), the IC<sub>50</sub> value obtained was 33.36  $\mu$ M, whereas for compound **8** obtained after the elimination of the  $\alpha$ -methylene- $\gamma$ -butyrolactone group the resulting IC<sub>50</sub> value was 20.32  $\mu$ M. In the case of isalantolactone (**1**) and compound **2**, they maintained a similar activity even after the removal of the aforementioned group. However, alantolactone (**4**) presented an IC<sub>50</sub> of 27.19  $\mu$ M and compound **5** of 57.77  $\mu$ M. When the hydroxyl group was introduced in the  $\alpha$  position to the lactone group, lappalone (**11**) and, especially, pertyolide A (**3**) suffered a big drop of activity unlike pertyolide B (**6**) which displayed a strong inhibitory activity.

### 3.2 Phytotoxic assay on *Lemna paucicostata*

The SLs with the  $\alpha$ -methylene- $\gamma$ -butyrolactone moiety in all cases presented strong inhibition of the proliferation of *L. paucicostata*, especially alantolactone (**4**) which exhibited an IC<sub>50</sub> value of 5.15  $\mu$ M (Table 2). Significant inhibition of the growth was observed at concentrations >10  $\mu$ M for alantolactone (**4**) and >33  $\mu$ M for isalantolactone (**1**) and dehydrocostuslactone (**7**), with advanced necrosis observed at



**Figure 2.** Effect of the compounds assayed on *L. sativa*. Values represent percentage differences in root elongation from the negative control.



**Figure 3.** Effect of the compounds assayed on *A. stolonifera*. Values represent percentage differences in germination rate from the negative control.

concentrations >100  $\mu\text{M}$ . However, in the case of the C<sub>17</sub>-sesquiterpenoids, pertyolides A (**3**) and B (**6**) and lappalone (**11**) did not show significant inhibition at concentrations <100  $\mu\text{M}$  and necrosis was observed only at the highest concentration tested (1000  $\mu\text{M}$ ).

**Table 1.** IC<sub>50</sub> values ( $\mu\text{M}$ ) obtained from the inhibition of the germination of *A. stolonifera*. Acifluorfen was used as positive control

Compound	IC <sub>50</sub> ( $\mu\text{M}$ )
<b>Acifluorfen</b>	3.97*
<b>1</b>	25.96*
<b>2</b>	24.81*
<b>3</b>	>10 000***
<b>4</b>	27.19*
<b>5</b>	57.77**
<b>6</b>	18.46*
<b>7</b>	33.36**
<b>8</b>	20.32**
<b>11</b>	53.25**

Statistically significant differences from the control: \*,  $P < 0.001$ ; \*\*,  $0.001 < P < 0.01$ ; \*\*\*,  $0.01 < P < 0.05$ .

**Table 2.** IC<sub>50</sub> values ( $\mu\text{M}$ ) obtained from the inhibition of the proliferation of *L. pausicostata*. Propanil was used as positive control

Compound	IC <sub>50</sub> ( $\mu\text{M}$ )
<b>Propanil</b>	3.10***
<b>1</b>	20.93*
<b>3</b>	247.85*
<b>4</b>	5.15***
<b>6</b>	228.11***
<b>7</b>	25.55*
<b>11</b>	120.98**

Statistically significant differences from the control: \*,  $P < 0.001$ ; \*\*,  $0.001 < P < 0.01$ ; \*\*\*,  $0.01 < P < 0.05$ .

**Table 3.** IC<sub>50</sub> values ( $\mu\text{M}$ ) obtained from the inhibition of *A. cepa*. Logran® was used as positive control

Compound	Root		Shoot	
	IC <sub>50</sub> ( $\mu\text{M}$ )	R <sup>2</sup>	IC <sub>50</sub> ( $\mu\text{M}$ )	R <sup>2</sup>
<b>Logran</b>	11.8	0.9162	15.2	0.9423
<b>1</b>	87.2	0.8596	600.2	0.7703
<b>2</b>	428.3	0.8417	918.9	0.8017
<b>3</b>	1209.0	0.9182	1317.0	0.9647
<b>4</b>	413.4	0.9574	208.0	0.9913
<b>5</b>	247.4	0.9041	170.8	0.9180
<b>6</b>	25.3	0.7995	64.4	0.8421
<b>8</b>	835.3	0.9842	782.7	0.9919
<b>11</b>	384.4	0.9310	627.4	0.8185
<b>14</b>	738.2	0.9880	-	-

No data is shown when 50% of inhibition was not achieved at the highest concentration tested. Compounds that did not reach 50% of inhibition for both root and shoot were omitted.

The presence of the  $\alpha$ -methylene- $\gamma$ -butyrolactone moiety seems to play an important role in the phytotoxic activity of the molecules against this species. The starting SLs presented a much stronger inhibitory activity compared to their corresponding C<sub>17</sub>-sesquiterpenoid, 50-fold higher in the most potent compounds alantolactone and pertyolide B, and  $\approx$ 5-fold higher in the case of dehydrocostuslactone and lappalone.

### 3.3 Phytotoxic assay on standard target species (STS) and target weeds

*Allium cepa* and *A. viridis* were two of the most sensitive species studied (Tables 3 and 4). Lappalone and its intermediates (**7–11**) were evaluated against the STS seeds and the results can be found in our previous work.<sup>19</sup> The activity of dehydrocostuslactone against *E. crus-galli* and *L. perenne* also can be found in the literature.<sup>34</sup>

#### 3.3.1 STS seeds

Only pertyolide C (**14**) from the guaianolide-type compounds (**12–14**) performed at best a weak inhibition activity against onion and tomato (Fig. 4). We can determine based on obtained IC<sub>50</sub> values of **12–14** and **7–10**<sup>19</sup> that introduction of the functionalization at position C-3 causes a significant decline in phytotoxic activity. However, good activity profiles were observed for the eudesmanolide-type molecules. Onion seeds were the most sensitive, followed by lettuce and tomato; almost no activity was observed against cress (Fig. 4; Tables 3, S1 and S2).

Pertyolide A (**3**) only showed moderate inhibition against onion with no significant activity for the other three seeds. Pertyolide B (**6**) exhibited strong inhibition against onion (IC<sub>50</sub>: 25.3 and 64.4  $\mu\text{M}$  versus root and shoot) and moderate inhibition of lettuce and tomato.

#### 3.3.2 Target weeds

The majority of the molecules (**1–11** and **14**) presented high inhibition (>90%) of *A. viridis* root and shoot length in the two or three highest concentrations (Figs 5 and 6; Table 4). A significant drop was observed for compounds **12** and **13**, both in root and shoot length, which supports the theory that the functionalization at C-3 lowers the activity as observed previously in some of the STS bioassays. However, this drop was observed only for the hydroxylated derivatives as pertyolide C (**14**) that presented a robust inhibition against *A. viridis* at a concentration >300  $\mu\text{M}$ .

**Table 4.** IC<sub>50</sub> values (μM) obtained from the inhibition of *A. viridis*

Compound	Root		Shoot		Germination rate	
	IC <sub>50</sub> (μM)	R <sup>2</sup>	IC <sub>50</sub> (μM)	R <sup>2</sup>	IC <sub>50</sub> (μM)	R <sup>2</sup>
<b>Logran</b>	33.2	0.9341	80.2	0.9799	193.5	0.9699
<b>1</b>	63.2	0.9342	60.3	0.9106	67.3	0.9184
<b>2</b>	123.2	0.9692	104.0	0.9511	229.6	0.9416
<b>3</b>	436.3	0.9395	316.0	0.9311	250.3	0.9729
<b>4</b>	46.1	0.9677	43.2	0.9468	53.1	0.8398
<b>5</b>	86.8	0.9764	63.0	0.9446	118.5	0.9379
<b>6</b>	56.7	0.9169	70.3	0.8394	24.0	0.8533
<b>7</b>	75.7	0.9718	93.9	0.9023	154.3	0.9328
<b>8</b>	54.1	0.9581	44.5	0.9164	77.8	0.9258
<b>9</b>	54.1	0.9868	54.4	0.9344	82.9	0.9350
<b>10</b>	133.6	0.9902	346.7	0.9658	84.9	0.9196
<b>11</b>	91.0	0.9612	103.8	0.9546	5.7	0.8898
<b>13</b>	732.7	0.9285	-	-	-	-
<b>14</b>	242.5	0.8495	210.4	0.8561	278.7	0.9211

Logran® was used as positive control. No data is shown when 50% of inhibition was not achieved at the highest concentration tested. Compounds that did not reach 50% of inhibition for root, shoot and germination rate were omitted.

Lappalone (**11**) and pertyolide B (**6**) presented the highest phytotoxic activity from the synthesized molecules against *A. viridis* with IC<sub>50</sub> values of 91.0, 103.8 and 5.7 μM against root and shoot elongation, and germination rate (respectively) for lappalone and 56.7, 70.3 and 24.0 μM against root and shoot elongation, and germination rate (respectively) for pertyolide B. In the case of *M. maximus*, the eudesmanolides-type SLs (Fig. 7; Table S3) presented a strong inhibition at the two highest concentrations except for pertyolide A (**3**) which did not show significant activity. Pertyolide B (**6**) displayed IC<sub>50</sub> values of 195.5 and 165.3 μM against root and shoot elongation. Dehydrocostuslactone (**7**) and compounds **8** and **11** from the guaianolides tested displayed the best phytotoxicity activity against this seed (Fig. 6; Table S3). Dehydrocostuslactone showed an inhibition against root and shoot with values >90% at 1000 and 300 μM, whereas compound **8** strongly inhibited the root at the two highest concentrations tested and compound **11** presented good inhibition values, especially, against shoot with inhibitions >50% in the concentration range from 100 to 1000 μM. Compounds **9–10** only showed moderate profiles and no significant activity was observed for **12–14**.

Lappalone (**11**) and compounds **1, 4** and **10** presented the best activity against *E. crus-galli* (Figs 5 and 8); in particular, lappalone strongly inhibited shoot growth with an IC<sub>50</sub> value of 5.0 μM (Table S4). The rest of the molecules presented rather a moderate profile of activity with some peaks of activity at the highest concentrations. Lappalone was the only guaianolide that showed a good inhibition profile against *L. perenne* (Fig. 8; Table S5). The eudesmanolides: **1, 4** and **5** presented strong inhibition values, highlighting the results obtained against shoot growth of >80% at 1000 μM and ≈60% at 300 μM (Fig. 7; Table S5). The rest of the molecules exhibited a moderate inhibition profile against this seed.

The germination rate of the selected weed species also was evaluated. This variable was only significantly affected in the case of *A. viridis* (Fig. 9 and Table 4). The high inhibition of the germination rate of *A. viridis* seeds was observed in the majority of the

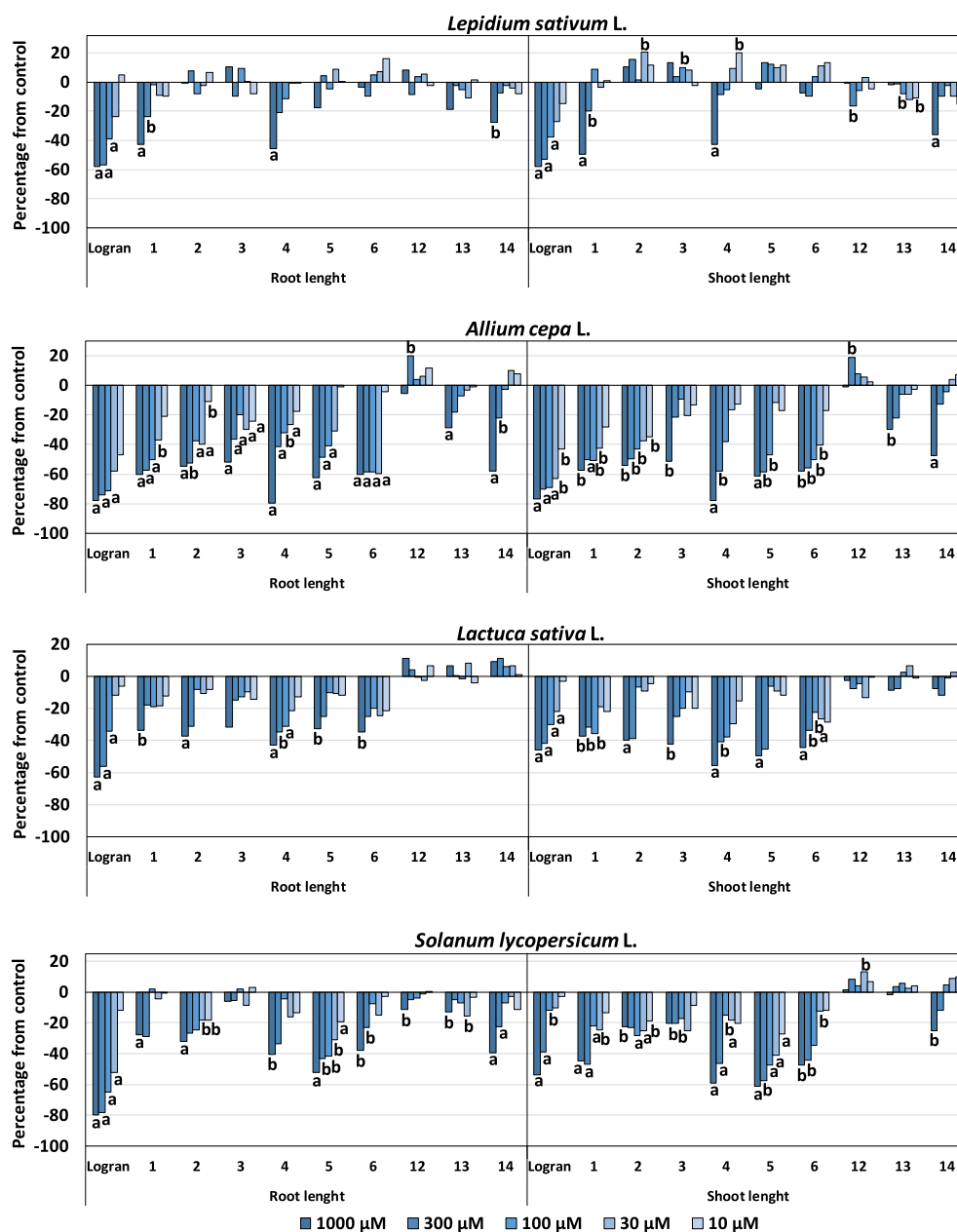
studied molecules except for compounds **12–14**. The best inhibition profiles were obtained for lappalone (**11**) and pertyolide B (**6**), with IC<sub>50</sub> values of 5.7 and 24.0 μM, respectively.

In summary, lappalone (**11**) and pertyolide B (**6**) presented the best overall phytotoxic activity against selected weed species. Based on the results obtained in the different assays, we can observe that the introduction of the functionalization at the position C-3 (**12–14**) prompts a decrease in the activity of the guaianolides when compared to their nonmodified counterparts (**7–11**). It is well-known that the physicochemical properties of molecules (e.g. aqueous solubility, polarity, electronic distribution, steric factors) play an important role in their capability of being absorbed and transported through cell membranes to their site of action. It is possible that the addition of the hydroxyl group at C-3 may produce an important change in any of these properties that limits the successful transport of the molecule through the living system to the site of action reducing its activity.<sup>35</sup>

In many cases, the assayed compounds presented much higher phytotoxic activity against the selected weeds than the crops used in the STS assay. Some clear examples can be observed against *A. viridis* where a strong inhibition (>80%) was detected at concentrations >100 μM for pertyolide B (**6**) and 300 μM for pertyolide C (**14**), whereas no significant phytotoxicity was observed against *L. sativum*, *L. sativa* and *S. lycopersicum* at the aforementioned concentrations. The selectivity exhibited by some of the molecules could be exploitable for the development of a post-emergence herbicide.

### 3.4 Broomrape seed germination assay

The assayed compounds stimulated only the germination of *P. ramosa* (Fig. 10); no significant stimulation was observed against *O. cumana* and *O. crenata*. It has been reported previously that the activity of dehydrocostuslactone (**7**) moderately stimulated the germination of *O. cumana*.<sup>34</sup> However, none of the derivatives synthesized from it presented any activity against this seed. It is well-known that parasitic plants are selective for the



**Figure 4.** Results obtained in the STS for compounds **1–6** and **12–14**. Values represent percentage differences in root and shoot elongation from the negative control. Logran<sup>®</sup> was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .

kind of natural compound produced by their host species that stimulates their germination.

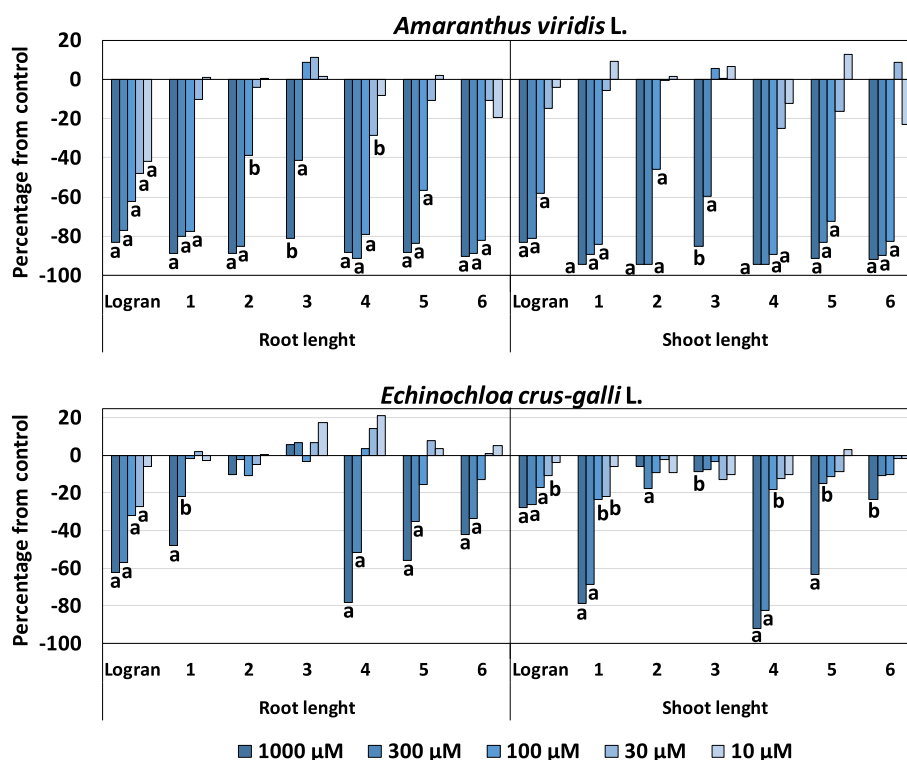
Lappalone (**11**) presented the best stimulation profile for *P. ramosa* reaching stimulation values  $>50\%$  for concentration levels  $>1.0 \mu\text{M}$ . Overall, the guaianolide-type molecules exhibited better stimulation profiles than the eudesmanolide-type, with some of them reaching stimulation levels of  $\approx 50\text{--}60\%$  of the seeds at 10 or  $1.0 \mu\text{M}$ , whereas the other type managed to stimulate only  $\approx 40\%$  of the seeds at  $100 \mu\text{M}$  except isoalantolactone (**1**). It is important to remark that the guaianolides with the hydroxyl group at position C-3 (**12** and **13**) presented really low stimulatory activity ( $<20\%$  at the highest concentration tested) and peryolide C (**14**) only presented strong activity at  $100 \mu\text{M}$  whereas compounds **8–11** managed to stimulate the germination of 50% of the seeds at concentrations  $>10$  and  $1.0 \mu\text{M}$ . Similar to the results

obtained in the phytotoxicity assays, these results suggest that the functionalization at the mentioned position hinders the activity of these molecules in stimulating the germination of *P. ramosa*.

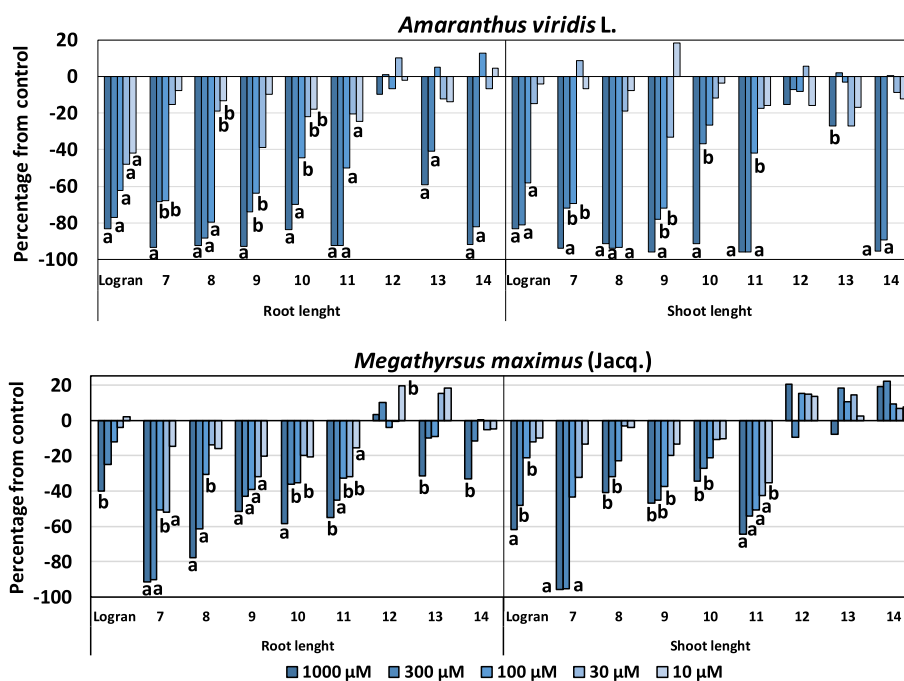
### 3.5 Antifungal assay

First, none of the compounds evaluated in a primary assay inhibited the growth of *B. cinerea*. However, they affected the growth of *C. fragariae* and *F. oxysporum* (Table 5).

From all of the compounds assayed, none of them showed stronger inhibitory activity than the commercial fungicides used as positive controls (benomyl and azoxystrobin against *C. fragariae* and fludioxonil, chlorothalonil, dodine, thiram and prochloraz against *F. oxysporum*). Overall, alantolactone (**4**) and dehydrocostuslactone (**7**) exhibited the strongest activity against both fungi species. The removal of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system (**5** and **8**)



**Figure 5.** Results obtained for compounds 1–6 against *A. viridis* and *E. crus-galli*. Values represent percentage differences in root and shoot elongation from control. Logran® was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .

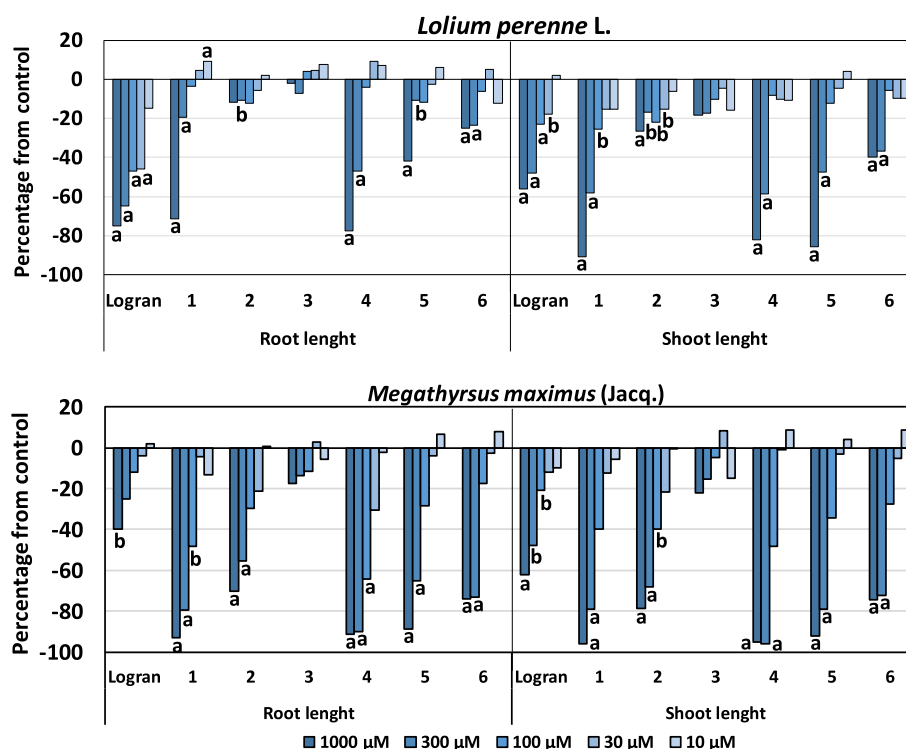


**Figure 6.** Results obtained for compounds 7–14 against *A. viridis* and *M. maximus*. Values represent percentage differences in root and shoot elongation from control. Logran® was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .

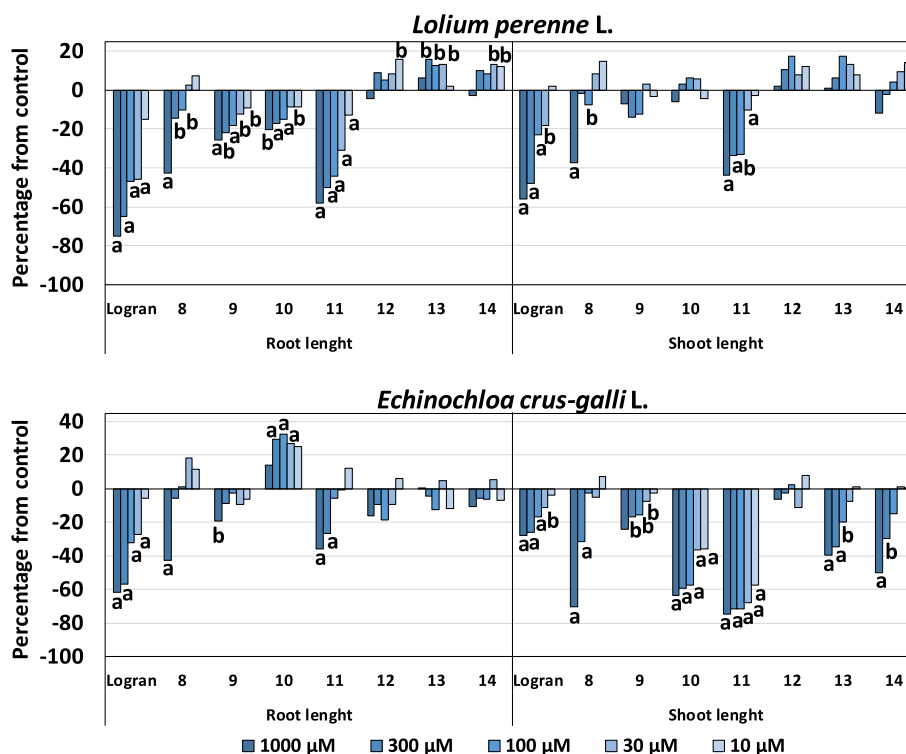
eneebles the inhibitory properties against *C. fragariae*, resulting in a smaller and diffused areas; however, no significant difference was observed against *F. oxysporum*. Isoalantolactone (**1**) was not as effective as **4** and **7**, and its activity was completely lost after the elimination of the butyrolactone group (**2** and **3**). The introduction of the

hydroxyl group in the  $\alpha$  position to the lactone group had different effects depending on the studied molecule. Its addition caused the complete loss of the activity of lappalone (**11**), yet that of pertyolide B (**6**) reached similar or even slightly better inhibition areas when compared to alantolactone (**4**).





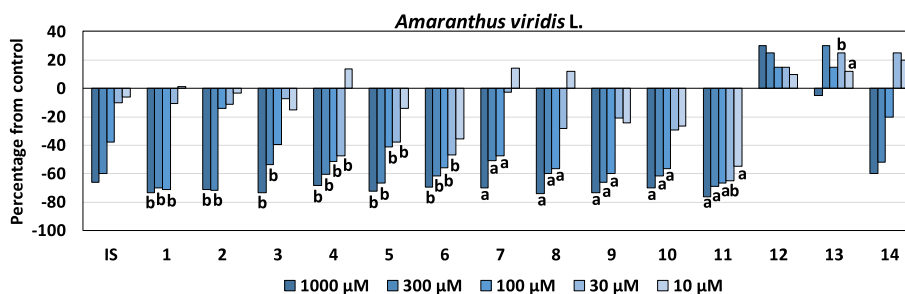
**Figure 7.** Results obtained for compounds 1–6 against *L. perenne* and *M. maximus*. Values represent percentage differences in root and shoot elongation from control. Logran® was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .



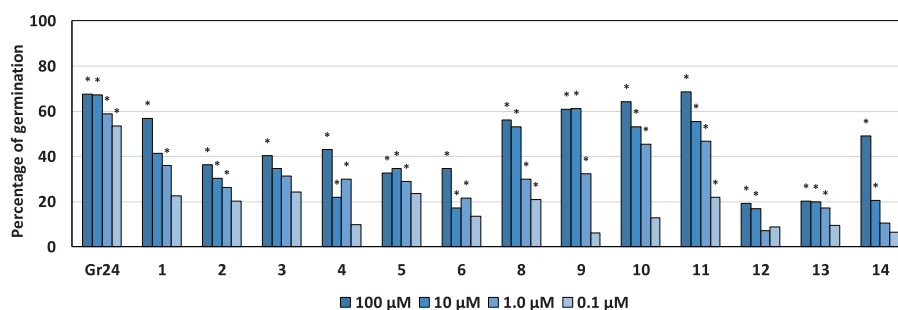
**Figure 8.** Results obtained for compounds 8–14 against *L. perenne* and *E. crus-galli*. Values represent percentage differences in root and shoot elongation from control. Logran® was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .

Subsequently, the most active molecules (4–8) were assessed in a secondary assay to determine their minimum inhibitory concentration (MIC). The MIC was determined as the minimum concentration of the chemical which produced clear inhibition zones (Table 6).

In a similar way to the primary assay, there was a general descent of activity between the SLs (4 and 7) and 5, 6 and 8 suggesting that the presence of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system contributes to the antifungal activity of the molecule



**Figure 9.** Effect of compounds 1–14 on the germination rate of *A. viridis*. Values represent percentage differences in root and shoot elongation from control. Logran® was used as positive control. Statistical significance from the control: 'a',  $P < 0.01$ ; 'b',  $0.01 < P < 0.05$ .



**Figure 10.** Stimulatory activity of the compounds assayed on *P. ramosa*. The synthetic strigolactone Gr24 was used as positive control. Significant differences from the negative control: \*,  $P < 0.05$ .

**Table 5.** Effect of the assayed compounds in the growth of *C. fragariae* and *F. oxysporum*

Compound	<i>C. fragariae</i>		<i>F. oxysporum</i>	
	100 μg	20 μg	100 μg	20 μg
1	7 <sup>b</sup>	3 <sup>b</sup>	6 <sup>b</sup>	2 <sup>b</sup>
2	-	-	-	-
3	-	-	-	-
4	10 <sup>a</sup>	6 <sup>a</sup>	7 <sup>a</sup>	3 <sup>a</sup>
5	4 <sup>a</sup>	-	7 <sup>a</sup>	3 <sup>a</sup>
6	9 <sup>a</sup>	5 <sup>a</sup>	9 <sup>a</sup>	4 <sup>a</sup>
7	13 <sup>a</sup>	9 <sup>a</sup>	7 <sup>a</sup>	3 <sup>a</sup>
8	7 <sup>b</sup>	3 <sup>b</sup>	7 <sup>a</sup>	3 <sup>a</sup>
11	-	-	-	-

The diameter of the inhibition zones observed are shown in mm (mean of three replicates). Benomyl and azoxystrobin were used as positive control against *C. fragariae* (dose applied 1.16 and 1.61 μg, and inhibition zones observed 15 and 19 mm, respectively) whereas fludioxonil, chlorothalonil, dodine, thiram and prochloraz were used as positive control against *F. oxysporum* (dose applied 1.00, 1.06, 1.15, 0.96 and 1.51 μg, and inhibition zones observed 8, 10, 10, 5 and 10 mm, respectively).

The aspect of the zones is represented as 'a' for clear zones and 'b' for diffuse zones.

**Table 6.** MIC (μg μL<sup>-1</sup>) determined against *C. fragariae* and *F. oxysporum* for compounds 4–8

Compound	<i>C. fragariae</i>	<i>F. oxysporum</i>
4	2.00	6.66
5	6.66	6.66
6	6.66	6.66
7	2.00	2.00
8	20.00	6.66

against the tested species. The reduction was more significant in the case of the guaianolides. However, the presence of the mentioned moiety does not seem to be essential for the antifungal activity against *F. oxysporum*, as observed in the results obtained for 4–6. No significant change of activity was observed against *F. oxysporum* after the elimination of the butyrolactone system or the introduction of the hydroxyl group. This suggests that other properties of the molecules also may play an important role in the antifungal activity. Another example can be observed in the obvious differences between isoalantolactone (1), pertyolide A (3) and

compound **2** when compared to alantolactone (**4**), dehydrocostuslactone (**7**) and their respective counterparts.

## 4 CONCLUSIONS

In this study, we evaluated the potential of four natural C<sub>17</sub>-sesquiterpenoids as pest control agents. Lappalone (**11**) and pertyolide B (**6**) presented the most promising results among them: **11** exhibited good activities against *A. viridis* and *E. crus-galli* and a good stimulation of the germination of *P. ramosa*, whereas **6** exhibited strong activities against *A. cepa* (model seed for the Liliaceae family), *A. viridis* and *M. maximus*, and a moderate inhibition of lettuce and tomato (representatives of the Asteraceae and Solanaceae families, respectively). In addition, **6** also presented good inhibition of the growth of the phytopathogenic fungi *C. fragariae* and *F. oxysporum*. Pertyolides A (**3**) and C (**14**) showed significantly less activity with only signalled cases where they presented moderate to high activity.

The results obtained through these studies confirm that the presence of the  $\alpha$ -methylene- $\gamma$ -butyrolactone system is important for the activity of SLs, as many of them have demonstrated strong activity in many of the assays performed in this work. However, its presence is not strictly necessary: there were many cases of the target molecules, as well as intermediates, without this moiety that presented similar or even higher activity than the starting SLs. To date, there is no study regarding the MoA of C<sub>17</sub>-sesquiterpenoids, and the results obtained with this study should encourage further research in this regard as these molecules could lead to new and alternative MoAs different from the usual Michael addition known for SLs.

Based on the results observed, it is possible to determine that the functionalization at C-3 on the guaiane-type SLs can cause (in some cases) a big drop in their activity. This was visible in the results obtained against *A. viridis*, *E. crus-galli* (shoot), *M. maximus*, *P. ramosa* and *S. lycopersicum*. It is possible that this modification may alter the physicochemical properties of the molecules which play an important role in their absorption and transport through living organisms, limiting their capability to reach their site of action and perform their bioactivity.

## ACKNOWLEDGEMENT

This research was supported by the Ministerio de Economía, Industria y Competitividad (MINEICO), Spain, PID2020-11574RB-I00/AEI/10.13039/501100011033. Thanks to FITÓ, Leonardo Velasco and Maurizio Vurro for generously providing the seeds used in the bioassays.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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