Evaluation of the phytotoxic and antifungal activity of C_{17}-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_{17}-sesquiterpenoids and performed a study of the effect caused by the elimination of the α-methylene-γ-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_{50}) value of 5.0 μM against Echinochloa crus-galli L. shoot and 5.7 μM 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_{50}: 56.7, 70.3 and 24.0 μM against the root and shoot growth, and germination rate, respectively) and Allium cepa L. (representative of the Liliaceae family, with IC_{50} values of 25.3 and 64.4 μM 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 μg mL^{-1}.

CONCLUSION: The bioassays revealed that frequently the presence of the α-methylene-γ-butyrolactone system is not essential for the bioactivities of sesquiterpene lactones, and suggest that C_{17}-sesquiterpenoids may function through a different mechanism of action not related to the widely assumed Michael addition.

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

Keywords: C_{17}-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.1 Worldwide, >200 active ingredients have been registered for herbicide use, but only 29 modes of action (MoA) have been reported.2 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 agrochemicals3 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.4,5

Sesquiterpene lactones (SLs) are one of the largest family of natural products and a broad range of bioactivities already have been...
reported. SLs are isolated mainly from the aerial parts of plants of the Compositae family, although they also can be found in other plant families, such as Umbelliferae, Lauraceae and Magnoliaceae. A biosynthesis of SLs with high phytotoxic and antifungal activity, by many weed species generated interest in their possible application for pest management. 

Recent isolation studies have led to the discovery of a novel family of SLs commonly named C_{17}-sesquiterpenoids. These compounds possess an unusual carbonated skeleton of 17 units and have eliminated the α-methylene-γ-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.

Up-to-date studies on the biological activities of C_{17}-sesquiterpenoids are very limited. Only a few medical activities have been reported; however, no information on their application in agriculture can be found. In our previous reports, we noticed that some of the C_{17}-sesquiterpenoids, as well as some intermediates synthesized without the α-methylene-γ-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_{17}-sesquiterpenoids [lappalene (1) and pertyolides A (3), B (6) and C (14)], as well as three natural SLs [dehydrocostuslactone (7), isoacontolactone (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 α-methylene-γ-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.

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 Lemma paucicostata L., a monocotyledonous species widely used to evaluate the toxicology of xenobiotic compounds to aquatic organisms.

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). Furthermore, the compounds also were assayed against four target weed seeds (Lolium perenne L., Echinochloa crus-galli L., Amburanthus viridis L. and Megathyrsus maximus Jacq.).

Parasitic plants are a special type of weed. They depend on a host plant to acquire nutrients and subsists. 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 administrated 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.

In this assay we study the potential of the compounds to stimulate the germination of three parasitic plants (Orobanche cumana Wallr., Orobanche 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: Collechotrichum fragariae Brooks., Fusarium oxysporum Schlechtend.: Fr. and Botrytis cinerea Pers.: F.

2 MATERIALS AND METHODS

2.1 Isolation and synthesis of the tested molecules

Isoacontolactone (1) and alantolactone (4) were isolated by column chromatography from Inula helenium roots and...
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.\(^\text{19,20}\)

### 2.2 Seeds and fungi origin

*S. lycopersicum*, *L. sativa* (cv Romana), *A. cepa* and *L. sativum* seeds were generously provided by FITO, *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 Istituto 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 x ananassa* Duchesne), *F. oxysporum* was isolated from orchid (*Cynoches* sp.) and *B. cinerea* was isolated from grape (*Vitis vinifera* L.).\(^\text{26}\) 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.\(^\text{27}\) 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, 33.0 μM, 100.0 μM, 33.0 μM and 10.0 μM). 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\(_{50}\)) 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.\(^\text{27,28}\) 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, 33.0 μM, 100.0 μM, 33.0 μM, 10.0 μM, 3.3 μM, 1.0 μM and 0.33 μM). 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\(_{50}\)) 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.\(^\text{19}\) 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\(^\text{\textsuperscript{a}}\) 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\(_{50}\) values using *Prism* v5.00 (GraphPad, San Diego, CA, USA).\(^\text{29}\)

### 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.\(^\text{30}\) 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. fragareae*, *F. oxysporum* and *B. cinerea*) following the thin layer chromatography (TLC) bioautography procedure described in the literature.\(^\text{31,32}\) The effectiveness of the chemicals was measured by analyzing the diameter and the aspect (clear of 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 μg of the compounds were spotted on triplicate on the TLC plates (Uniplate\textsuperscript{TM} Silica Gel GHFL Scored 10 × 20 cm, 250 μm). In the secondary assay, 100.00 μg, 33.33 μg, 10.00 μg, 3.33 μg, 1.00 μg and 0.33 μg of the compounds were spotted by triplicate on the TLC plates. The commercial fungicides benomyl, azoxystrobin, fluoxdioxonil, chlorothalonil, dodine, thiiram 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 isosalantolactone (1) and alantolactone (4) presented strong inhibition activity at concentrations >1 mM and 330 μM, respectively, with a huge drop in their activity at the lower concentrations. The elimination of the α-methylene-γ-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...
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 α-methylene-γ-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 μM. It is interesting to remark that in the case of compounds 5 and 8 we could observe cases of hormesis, 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 IC₅₀ value obtained was 33.36 μM, whereas for compound 8 obtained after the elimination of the α-methylene-γ-butyrolactone group the resulting IC₅₀ value was 20.32 μM. In the case of isoalantolactone (1) and compound 2, they maintained a similar activity even after the removal of the aforementioned group. However, alantolactone (4) presented an IC₅₀ of 27.19 μM and compound 5 of 57.77 μM. When the hydroxyl group was introduced in the α 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 pausicostata

The SLs with the α-methylene-γ-butyrolactone moiety in all cases presented strong inhibition of the proliferation of L. pausicostata, especially alantolactone (4) which exhibited an IC₅₀ value of 5.15 μM (Table 2). Significant inhibition of the growth was observed at concentrations >10 μM for alantolactone (4) and >33 μM for isoalantolactone (1) and dehydrocostuslactone (7), with advanced necrosis observed at

![Figure 2](image1.png)

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

![Figure 3](image2.png)

**Figure 3.** Effect of the compounds assayed on A. stolonifera. Values represent percentage differences in germination rate from the negative control.
concentrations >100 μM. However, in the case of the C17-sesquiterpenoids, pertyolides A (3) and B (6) and lappalone (11) did not show significant inhibition at concentrations <100 μM and necrosis was observed only at the highest concentration tested (1000 μM).

The presence of the α-methylene-γ-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 C17-sesquiterpenoid, 50-fold higher in the most potent compounds alantolactone and pertylide B, and ≈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.19 The activity of dehydrocostuslactone against E. crus-galli and L. perenne also can be found in the literature.34

#### 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 IC50 values of 12–14 and 7–1011 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 (IC50: 25.3 and 64.4 μM 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 μM.

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### Table 1. IC50 values (μM) obtained from the inhibition of the germination of A. stolonifera. Acifluorfen was used as positive control

| Compound | IC50 (μM) |
|----------|-----------|
| Acifluorfen | 3.97* |
| 1 | 25.96* |
| 2 | 24.81* |
| 3 | >10 000*** |
| 4 | 27.19* |
| 5 | 57.77*** |
| 6 | 18.46* |
| 7 | 33.36** |
| 8 | 20.32** |
| 11 | 53.25** |

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

### Table 2. IC50 values (μM) obtained from the inhibition of the proliferation of L. pausescostata. Propanil was used as positive control

| Compound | IC50 (μM) |
|----------|-----------|
| Propanil | 3.10*** |
| 1 | 20.93* |
| 2 | 247.85 |
| 4 | 5.15*** |
| 6 | 228.11*** |
| 7 | 25.55* |
| 11 | 120.98** |

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

### Table 3. IC50 values (μM) obtained from the inhibition of A. cepa. Logran* was used as positive control

| Compound | Root | Shoot |
|----------|------|-------|
| Logran | IC50 (μM) | R² | IC50 (μM) | R² |
| 1 | 11.8 | 0.9162 | 15.2 | 0.9423 |
| 2 | 87.2 | 0.8596 | 600.2 | 0.7703 |
| 3 | 428.3 | 0.8417 | 918.9 | 0.8017 |
| 4 | 1209.0 | 0.9182 | 1317.0 | 0.9647 |
| 5 | 413.4 | 0.9574 | 208.0 | 0.9913 |
| 6 | 247.4 | 0.9041 | 170.8 | 0.9180 |
| 7 | 25.3 | 0.7995 | 64.4 | 0.8421 |
| 8 | 835.3 | 0.9842 | 782.7 | 0.9919 |
| 11 | 384.4 | 0.9310 | 627.4 | 0.8185 |
| 14 | 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.

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Table 4. IC50 values (μM) obtained from the inhibition of A. viridis

| Compound | Root IC50 (μM) | Root R² | Shoot IC50 (μM) | Shoot R² | Germination rate IC50 (μM) | Germination rate R² |
|----------|----------------|---------|----------------|---------|---------------------------|---------------------|
| Logran   | 33.2           | 0.9341  | 80.2           | 0.9799  | 193.5                     | 0.9699              |
| 1        | 63.2           | 0.9342  | 60.3           | 0.9106  | 67.3                      | 0.9184              |
| 2        | 123.2          | 0.9692  | 104.0          | 0.9511  | 229.6                     | 0.9416              |
| 3        | 436.3          | 0.9395  | 316.0          | 0.9311  | 250.3                     | 0.9729              |
| 4        | 46.1           | 0.9677  | 43.2           | 0.9468  | 53.1                      | 0.8398              |
| 5        | 86.8           | 0.9764  | 63.0           | 0.9464  | 118.5                     | 0.9379              |
| 6        | 56.7           | 0.9169  | 70.3           | 0.8394  | 24.0                      | 0.8533              |
| 7        | 75.7           | 0.9718  | 93.9           | 0.9023  | 154.3                     | 0.9328              |
| 8        | 54.1           | 0.9581  | 44.5           | 0.9164  | 77.8                      | 0.9258              |
| 9        | 54.1           | 0.9868  | 54.4           | 0.9344  | 82.9                      | 0.9350              |
| 10       | 133.6          | 0.9902  | 346.7          | 0.9658  | 84.9                      | 0.9196              |
| 11       | 91.0           | 0.9612  | 103.8          | 0.9546  | 5.7                       | 0.8898              |
| 12       | 732.7          | 0.9285  | -              | -       | -                         | -                   |
| 13       | 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 synthetized molecules against A. viridis with IC50 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 IC50 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 IC50 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 IC50 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.

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. sativa, L. sativum 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. 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...
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 μM. Overall, the guaianolide-type molecules exhibited better stimulation profiles than the eudesmanolide-type, with some of them reaching stimulation levels of ≈50–60% of the seeds at 10 or 1.0 μM, whereas the other type managed to stimulate only ≈40% of the seeds at 100 μM 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 pertyolide C (14) only presented strong activity at 100 μM whereas compounds 8–11 managed to stimulate the germination of 50% of the seeds at concentrations >10 and 1.0 μM. 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. fragareae 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 azoxystrubub against C. fragareae and fluifoxonil, 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 α-methylene-γ-butyrolactone system (5 and 8)
enfeebles the inhibitory properties against C. fragareae, 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 α 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).
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 α-methylene-γ-butyrolactone system contributes to the antifungal activity of the molecule.

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’, $0.01 < P < 0.05$. 

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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

![Figure 9](image-url). 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](image-url). 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. fragareae* and *F. oxysporum*

| Compound | *C. fragareae* | *F. oxysporum* |
|----------|----------------|----------------|
|          | 100 μg         | 20 μg          | 100 μg         | 20 μg          |
| 1        | 7^b            | 3^b            | 6^b            | 2^b            |
| 2        | -              | -              | -              | -              |
| 3        | -              | -              | -              | -              |
| 4        | 10^a           | 6^a            | 7^a            | 3^a            |
| 5        | 4^a            | 5^a            | 7^a            | 3^a            |
| 6        | 9^a            | 9^a            | 7^a            | 3^a            |
| 7        | 13^b           | 9^a            | 7^a            | 3^a            |
| 8        | 7^b            | 3^b            | 7^a            | 3^a            |
| 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. fragareae* (dose applied 1.16 and 1.61 μg, and inhibition zones observed 15 and 19 mm, respectively) whereas fluinoxonil, 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⁻¹) determined against *C. fragareae* and *F. oxysporum* for compounds 4–8

| Compound | *C. fragareae* | *F. oxysporum* |
|----------|----------------|----------------|
| 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           |
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 C17-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. fragarum 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 α-methylene-γ-butylolactone 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 C17-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 functionalization at C-3 on the guaiane-type SLs can cause significant drop in their activity. This was visible in the

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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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