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Phytotoxic Effect of Macerates and Mulches from *Cupressus leylandii* Leaves on Clover and Cress: Role of Chemical Composition

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Abstract: The use of plant secondary metabolites is an attractive strategy to control weeds. In this work, cypress (*Cupressus leylandii*) leaves were collected and tested as aqueous macerates or mulches for their ability to control seed germination and seedling growth of *Trifolium repens* and *Lepidium sativum*. Leaves were collected on trees facing the north (S) or facing the sun (L). The rate of seed germination measured on sand was drastically slowed down by the cypress leaves after 8 d of maceration, reaching inhibition of >85% compared to the control at 7 d post-imbibition (dpi). Analysis of macerates by UHPLC-MS revealed the presence of organic acids, phenols, and sugars in amounts increasing with maceration time and the phytotoxic effect. A 5 cm layer of cypress leaf mulch also significantly reduced ($p < 0.001$) the rate of seedling appearance of *Lepidium sativum* measured on potting soil compared to the control. Mulches prepared using L leaves were more efficient than those prepared with S leaves ($p = 0.0029$). Analysis of ethanolic extracts of leaves by mass spectrometry (MS) coupled to liquid (UHPLC) or gas chromatography (GC) showed the presence of a variety of monoterpenes, monoterpenoids, and diterpenoids with a labdane backbone. They were all more concentrated in mulches prepared with L leaves than those prepared with S leaves, in particular diterpenoids, which were about 10-fold more concentrated. However, the identification of phytotoxic components needs further research. It is concluded that due to their phytotoxic properties, *Cupressus leylandii* leaves could be used as mulch or macerate for target treatment of weedy areas.

Keywords: antigerminative; allelopathy; cypress; extracts; mass spectrometry; labdane-derived diterpenoids

1. Introduction

Using plants to control weeds is an attractive strategy that has the advantage of reducing the use of synthetic herbicides and their deleterious environmental impact and is a valuable form of green waste recycling [1]. Research in this field started to grow with the discovery that some plants can negatively affect other plants in their vicinity. Two explanations for the phenomenon are proposed: competition for plant resources and/or generation of phytotoxic secondary metabolites, named allelochemicals. Allelochemicals are expected to be released in the rhizosphere and expand through leaching in the soil or volatilization to prevent the germination or growth of weed seedlings present in the immediate vicinity [2]. The finding that many secondary metabolites exhibit phytotoxicity in laboratory conditions reinforced this hypothesis [3]. Phytotoxic secondary metabolites belong to a variety of chemical classes, but are mainly phenols and terpenoids [4–11].

Another way to take advantage of the phytotoxicity of plant secondary metabolites is to incorporate plant wastes into the soil as green manure [12] or apply them as mulch [13,14]. Mulches are expected

to inhibit seed germination and weed growth in two ways: they can release phytotoxic secondary metabolites that disseminate into the soil by leaching and reach the recipient weed seeds and/or provide a physical barrier to light and moisture. Previous studies have shown that a variety of organic mulches, in particular pine bark, cypress, and hardwood mulches, are effective in weed suppression [15–17].

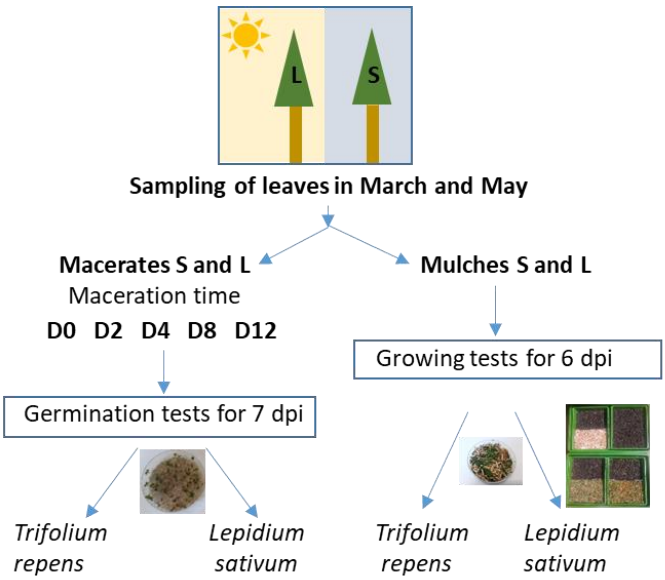
The present work focused on *Cupressus leylandii*. These trees are largely used as hedges in municipal or private gardens and can yield significant amounts of green waste. Yet, their capacity to control weeds is poorly documented in the literature. Moreover, leaves and wood of several other cypress species were reported to contain potential phytotoxic compounds: phenols [18], monoterpenoids [17–21], and diterpenoids [18–22].

We sampled leaves of *Cupressus leylandii* in trees facing the north or exposed to solar light during the spring period to investigate the effect of solar light on the phytotoxic activity. They were tested for their capacity to inhibit the seed germination of *Lepidium sativum* and *Trifolium repens* as macerates and control seedling growth as mulches. In parallel, the chemical compositions of macerates and ethanolic leaf extracts were analyzed to investigate potential links between phytotoxic properties and chemical contents.

2. Materials and Methods

2.1. Leaf Sampling

Fresh leaves of *Cupressus leylandii* were collected in March and May 2020. Leaves were collected from trees facing the north, protected from solar light exposure by a wall (sample S), and from trees facing the south, exposed to solar light (sample L). In general, the stock of L leaves contained more new leaves than the stock of S leaves, and the stock of leaves collected in May had more new leaves than the stock collected in March. Leaves were then stored in airtight plastic bags and kept in darkness at 15 °C. They were used for experiments two weeks following the sampling, as shown in Scheme 1.



Scheme 1. Summary of experimental conditions. Macerates and mulches prepared with cypress leaves collected on trees facing north (S) and south (L) were tested on *Trifolium repens* and *Lepidium sativum*.

2.2. Macerate Preparations

Leaves (50 g) were cut into 3–5 cm pieces and soaked in 250 mL of purified water in amber bottles at room temperature for up to 12 days. Only green parts were used. Incipient leaves were not selected, only mature leaves were taken. The bottles were closed to limit terpene volatilization but were opened for few seconds every day for reoxygenation. The macerated solutions were sampled after 4, 8, and 12

days (D4, D8, and D12, respectively) for leaves collected in March, and after 2, 4, and 8 days (D2, D4, and D8, respectively) for leaves collected in May. They were filtered on cellulose Whatman filters before chemical analysis or germination studies. Macerates prepared using L leaves at D2, D4, D8, and D12 were named L-D2, L-D4, L-D8, and L-D12, while those prepared with S leaves at D2, D4, D8, and D12 were named S-D2, S-D4, S-D8, and S-D12. The preparation of macerates was repeated three times.

2.3. Germination Tests with Macerates or Chemicals

Lepidium sativum (Radis et capucine, Bio, Botanic®) and *Trifolium repens* (Semences du Puy) were used for tests. A total of 25 seeds were placed in a Petri dish (9 cm diameter) on a sand bed (35 g) in 10 mL of purified water (control) or S and L macerates. They were placed in a thermostatically controlled room (23 ± 2 °C) for germination. All seeds were regarded as having germinated when the radicle had emerged and was 3 mm long. The number of germinated seeds was counted every 12 h for a maximum of 7 dpi. Four Petri dishes were used per condition and species. Germination assay with macerates at D8 and D12 was conducted three times. Coumaric acid (97%; Sigma-Aldrich) solutions prepared at 10^{-5} and 2.2×10^{-5} M were tested on *Lepidium sativum* and *Trifolium repens* in the same conditions as described for macerates. To test the effect of (E)Labd-13-ene-8,15-diol (Santa Cruz Biotechnology), we proceeded as follows. The molecule, which is poorly soluble in water, was dissolved in methanol at a concentration of 0.05 $g.mL^{-1}$, and 50 µL of this solution was added in droplets of 5 µL to the 10 mL of purified water directly in the Petri dishes. The final concentration of (E)Labd-13-ene-8,15-diol was of 10^{-3} M.

2.4. Seedling Growth Tests with Mulches

For this test, 25 seeds of *Lepidium sativum* or *Trifolium repens* were placed in a Petri dish (9 cm diameter) on a sand bed (35 g) in 10 mL of purified water. Seeds were covered with 1 cm of cypress mulch prepared by cutting L and S leaves collected in May into 2–3 cm pieces (mulch L and mulch S) or hemp mulch (Botanic®) used as a control. Mulches contained both green parts of leaves and small twigs. Incipient leaves were not selected, only mature leaves were taken. Three Petri dishes were used per condition. In another set of experiments, 4 × 25 seeds of *Lepidium sativum* were placed on potting soil, abundantly watered, and covered with 5 cm of S or L cypress mulch or hemp mulch (control). Seedlings able to go through the mulch layers were counted every day until 6 dpi.

2.5. Chemical Analysis

Macerates and ethanolic leaf extracts (2 g in 10 mL) prepared with leaves collected in May were analyzed by liquid chromatography coupled to high-resolution mass spectrometry (HRMS) on an Orbitrap Q-Exactive (Thermo Scientific) coupled to an Ultimate 3000 RSLC (Thermo Scientific) ultra-high-performance liquid chromatography (UHPLC) system equipped with an Acquity Phenomenex analytical column (2.1 mm × 100 mm, 1.7 µm particle size; Waters, USA). The aqueous solvent (A) consisted of a mixture of 0.1% formic acid, and the organic phase (B) was acetonitrile. Separation was achieved with a gradient program consisting of 0–7.5 min 5% and 7.5–8.5 min 99% of mobile phase B. After 8.5 min, the gradients were returned to the initial conditions and the analytical column was reconditioned for 3.5 min. The flow rate was set to 0.450 mL/min. The spectrometer operated in negative and positive modes. The formulas are proposed based on $ppm < 5$. Succinic acid and coumaric acid were identified and quantified by reference to authentics.

Ethanolic leaf extracts were analyzed by gas chromatography coupled to mass spectrometry (GC/EI-MS) using an Agilent 6890N Network gas chromatograph coupled to a 5973 Network mass selective detector and Agilent 7683B series injector. Data acquisition and processing and instrument control were performed by Agilent MSD ChemStation software. Compound separation was achieved by an HP-5MS (5% phenyl methyl siloxane) capillary column with 30 m length, 0.25 mm internal diameter, and 0.25 mm film thickness. The injector temperature was set at 250 °C and the injection

volume was 1.0 µL (split ratio of 20:1). The GC oven temperature program was: 50 °C for 1 min followed by a gradient of 10 °C min^{-1} to 250 °C and held for 5 min. Mass spectra were scanned between *m*/*z* 50 and *m*/*z* 500 with the source temperature set at 230 °C. Identification was based on matching query spectra to spectra present in the reference library (NIST17), with a minimum spectral similarity measure of 95%.

2.6. Germination Indices

Two germination indices were used to determine the effect of macerates and mulches on the kinetics of seed germination. In the case of macerates, the rate of germination (R) was defined as

$$R = N_1/1 + (N_2 - N_1)/2 + \ldots \ldots + (N_n - N_{n-1})/n \quad (1)$$

where $N_1, N_2, N_3, \ldots, N_n$ are the numbers of germinated seeds at 1, 2, 3, . . . , *n* days post-imbibition (dpi) and *n* is the number of days for monitoring [23]. Samples taken at 36, 60, 84, . . . dpi were treated as *n* = 1.5, 2.5, 3.5, . . . , etc. In the case of mulches, we used a similar index (Ra), but $N_1, N_2, N_3, \ldots, N_n$ are the numbers of seedlings appearing through the mulch layer with a height of around 5 cm at 1, 2, 3, . . . , *n* days post-imbibition. Ra is equal to

$$Ra = N_1/1 + (N_2 - N_1)/2 + \ldots \ldots + (N_n - N_{n-1})/n \quad (2)$$

2.7. Statistical Analysis

Germination tests and indices were compared to their respective controls using one-way ANOVA ($p < 0.05$) within each species. Data are given as mean ± SD. Tukey's honestly significant difference (HSD) test was used to compare treatment means two by two.

3. Results

3.1. Effect of Macerates on Seed Germination

Figure 1 shows the dynamics of germination of *Trifolium repens* and *Lepidium sativum* seeds in macerates prepared with leaves collected in May on trees facing the north (S) or exposed to solar light (L) and Table 1 the number of germinated seeds 7 days after imbibition. Figure 2 shows the rate of germination R measured at 7 and 5 dpi, while Table S1 gives the *p*-values calculated on R values using Tukey's test. For both seed species, macerates had a significant inhibiting effect on the kinetics of germination (Table S1). For *Trifolium repens*, the germination was delayed by 1.5 d compared to the control with macerates D2 and D4 and by a much longer time with macerate D8. No differences were found between macerates S and L at a given maceration time (Table S1). For *Lepidium sativum*, the germination was delayed by 2 d compared to the control with macerate D2, 3 d with macerate D4, and a much longer time with macerate D8. In this experimental condition, macerate L-D2 was significantly more efficient than macerate S-D2, with $p = 0.0051$, and L-D4 than S-D4, with $p = 0.037$.

Macerates prepared with leaves collected in March also inhibited seed germination (Table S2). The inhibition was complete with macerate D12 instead of macerate D8 for leaves collected in May.

The pH of macerates decreased during maceration from 5.9 at time 0 to 5.1 at D4 for macerate S and from 5.9 to 4.9 for macerate L. To investigate the pH effect on seed germination, we compared the effect of macerates S-D8 and L-D8 of May with and without neutralization using sodium bicarbonate. The same inhibition of *Lepidium sativum* seed germination by more than 95% was observed at 7 dpi for all treatments, suggesting that the acidity of macerates should not be involved in the inhibiting effect (Table 1).

Table 1. Number of germinated seeds for different treatments at 7 days post imbibition.

Treatment	Germinated Seeds	
	Trifolium Repens	*Lepidium Sativum*
Control	71 ± 8	98 ± 2
March, macerate S-D12	6 ± 2	4 ± 1
March, macerate L-D12	1 ± 1	1 ± 1
May, macerate S-D8	2 ± 1	1 ± 1
May, macerate S-D8 neutralized		1 ± 1
May, macerate L-D8	2 ± 1	2 ± 1
May, macerate L-D8 neutralized		1 ± 1

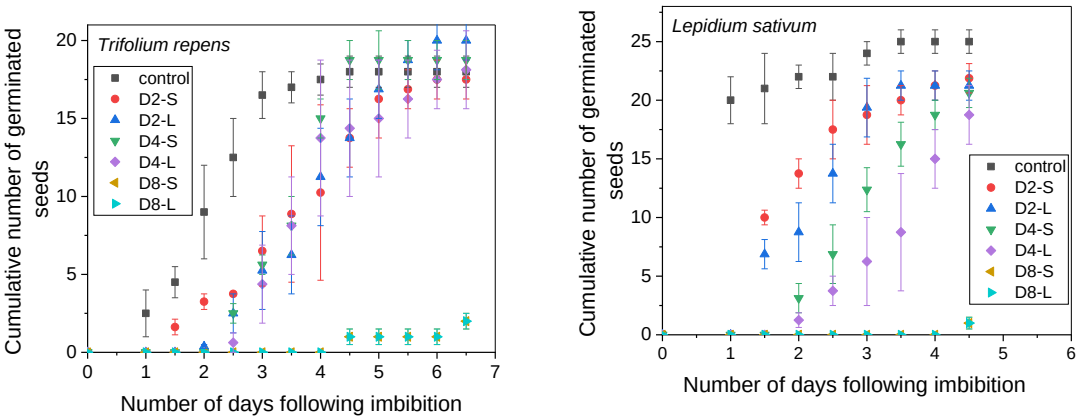


Figure 1. Germination dynamics of *Trifolium repens* (**left**) and *Lepidium sativum* (**right**) seeds in macerates prepared with leaves collected in May on trees facing north (S) or exposed to solar light (L). The germinated seeds are counted each day during the 7 days following the imbibition. Points represent mean values of four replicates (±standard error).

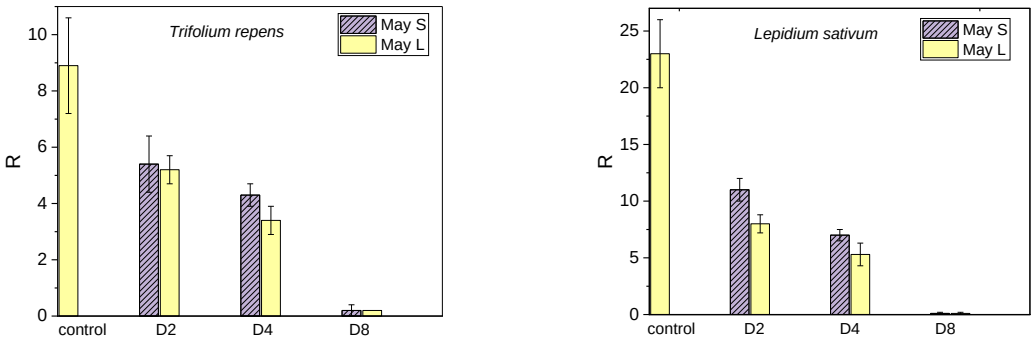


Figure 2. Rates of *Trifolium repens* (**left**) and *Lepidium sativum* (**right**) seed germination (R) measured at 7 and 5 dpi, respectively, in the presence of macerates prepared with leaves collected in May from trees facing north (S) or exposed to solar light (L). Mean values of four replicates (±standard error).

The results as a whole show that macerates inhibited the rate of germination of *Trifolium repens* and *Lepidium sativum*. The solar light exposure of trees increased the macerate phytotoxicity in the case of *Lepidium sativum*.

3.2. Effect of Mulch on Seedling Growth

Experiments in Petri dishes were conducted with 1 cm thick cypress mulch on both species for 6 d. The cypress mulch slowed down the appearance of seedlings compared to the controls. For *Trifolium repens*, at 6 dpi, 35 ± 2 and 40 ± 3% of seedlings appeared with mulches L and S, respectively, compared to 75% for the control. For *Lepidium sativum*, 50 ± 4 and 65 ± 5% of seedlings went through the mulch compared to 95% for the control. In the latter case, mulch L was little more efficient than mulch S ($p = 0.021$). In these experiments conducted in petri dishes, seeds are directly in contact with the chemicals released by the mulch. This is quite different from real conditions where soil constituents might adsorb the chemical released and/or microorganisms degrade them.

For this reason, another set of experiments was conducted in potting soil with 5 cm thick cypress mulch on *Lepidium sativum* seeds. The Ra index was used to evaluate the kinetics of the appearance of *Lepidium sativum* seedlings at 6 dpi through a 5 cm layer. Figure 3 compares the effects of a layer of hemp, mulch prepared with leaves collected in May from trees facing the north (S), and mulch prepared with leaves collected in May from trees exposed to solar light (L). Ra values were significantly lower for mulch S (3.71 ± 0.20) and mulch L (2.73 ± 0.40) than for the control hemp mulch (5.78 ± 0.24), with $p < 0.001$. The Ra value was also significantly lower ($p = 0.0029$) when measured with mulch L than mulch S.

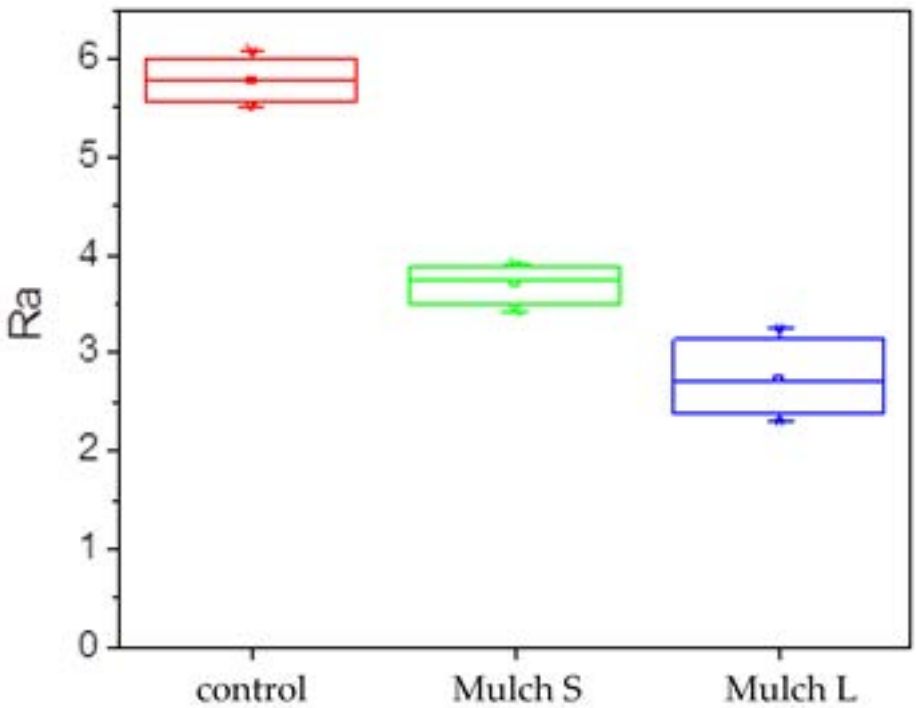


Figure 3. Compared kinetics of appearance (Ra) of *Lepidium sativum* seedlings at 6 days post imbibition through 5 cm layer of hemp and mulches prepared with leaves collected in May from trees facing north (S) or exposed to solar light (L). Mean values of four replicates (±standard error).

These experiments demonstrated that mulch prepared with cypress leaves could inhibit seed germination and/or seedling growth of *Lepidium sativum* in petri dished as in soil and that mulch L was more phytotoxic than mulch S.

3.3. Chemical Analysis

Macerates. UHPLC-HRMS analysis of macerates S and L prepared in May revealed the presence of at least 30 compounds (Figure S1, Table S3). The main ones appeared in the negative mode and belonged to the class of organic acids, phenols, and sugars. Peaks detected at m/z = 117.0182, 173.0448,

and 191.0552 in ESI⁻ were assigned to succinic ($C_4H_6O_4$), shikimic ($C_7H_{10}O_5$), and quinic ($C_7H_{12}O_6$) acids, respectively. The concentration of succinic acid was equal to 1.5×10^{-5} M in L-D2, 6.0×10^{-5} M in L-D4, and 1.3×10^{-4} M in L-D8, and 2.5×10^{-5} M in S-D2, 6.0×10^{-5} M in L-D4, and 1.6×10^{-4} M in S-D8. The peak at m/z = 163.0386 was attributed to coumaric acid; its concentration was within the range 10^{-6} to 10^{-5} M in the different samples. Peaks at m/z = 125.0232, 161.023, 287.0565, and 337.0934 were tentatively assigned to trihydroxybenzene ($C_6H_6O_3$), coumarine ($C_9H_6O_3$), a catechine derivative ($C_{15}H_{12}O_6$), and coumaroylquinic acid ($C_{16}H_{18}O_8$). The assignment of the compound with the formula $C_{16}H_{18}O_8$ to coumaroylquinic acid is consistent with the maximum of absorption at 311 nm as reported in the literature [24]. Two sugars, $C_7H_{14}O_7$ and $C_8H_{16}O_8$, were also detected. The UHPLC-HRMS peak area of almost all detected molecules increased with the maceration time. Illustration is given for quinic acid, coumaroyl quinic acid, and hydroxybenzoic acid in Figure 4. In Table 2, we present the sum of UHPLC-HRMS peak areas for the major classes of constituents in the different macerates. We can see that L macerates were a little more enriched in organic acids and sugars than S macerates, with peak area ratio, $Area_{L\text{-}DA}/Area_{S\text{-}D4}$, varying between 2.2 and 2.5 for D4, for instance (Table 2). Among phenols and phenolic acids, only coumaroylquinic acid was more concentrated in L than in S macerates (Figure 4).

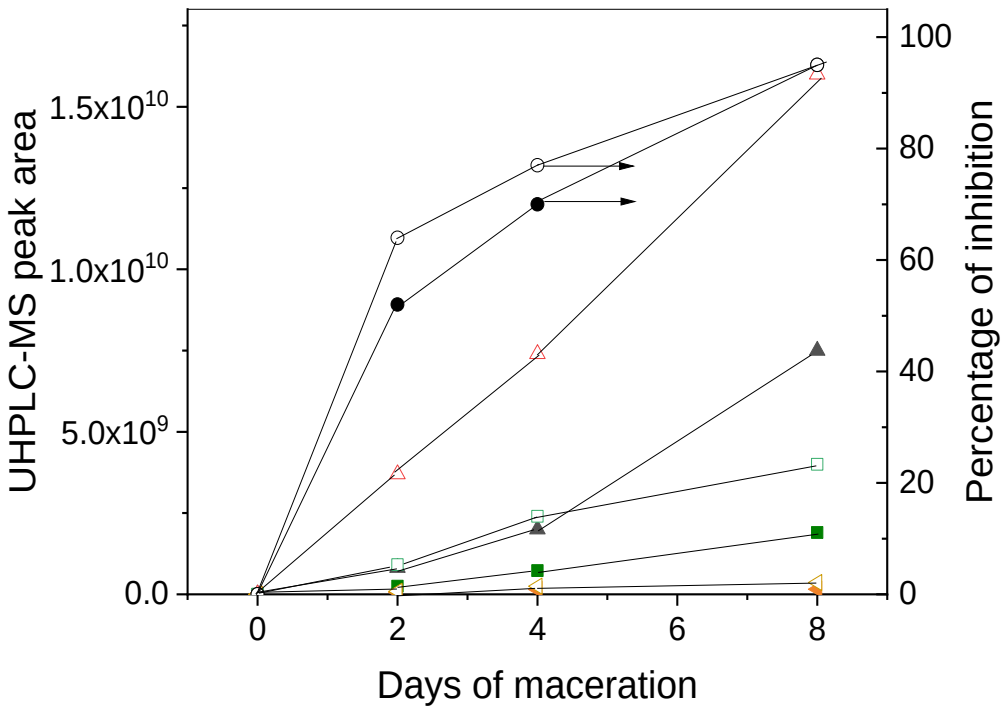


Figure 4. Effect of maceration time on UHPLC-MS peak area of quinic acid (△,▲), coumaroyl quinic acid (□,■), and hydroxybenzoic acid (◁,◀) (left *y*-axis) and on percentage of inhibition of *Lepidium sativum* seed germination (○,●) (right axis, arrows). Open symbols indicate macerates prepared with leaves collected from trees with solar exposure (L) and solid symbols indicate macerates prepared with leaves collected from trees facing north (S).

In the same family, coumaric acid has been reported to show phytotoxicity toward seed germination of several species [25,26]. We therefore conducted germination tests at coumaric acid concentrations of 10^{-5} and 2.2×10^{-5} M. No effect on the two species was observed at 10^{-5} M. Inhibition of *Trifolium repens* germination, however, was observed at 2.2×10^{-5} M (R = 7.7 ± 1.6 compared to 12.0 ± 0.5 for the control, p = 0.021).

Mulch. To characterize the less polar components of leaves, we made leaf extractions with ethanol and analyzed them by UHPLC-HRMS and GC-MS (Figure S2). UHPLC-HRMS analysis showed the expected presence of quinic and shikimic acids, along with catechine (m/z = 289.0721, $C_{15}H_{14}O_6$);

a peak at m/z = 537.0843, assigned to cupressoflavone [21]; and five diterpenoids (Table S4). The peaks at m/z = 301.2175 seemed to correspond to communic acid ($C_{20}H_{30}O_2$) and the one at 319.2285 to cupressic or isocupressic acid ($C_{20}H_{32}O_3$), two labdane diterpenoids already detected in other cypress species [20]. Moreover, we observed a compound at m/z = 299.2016 corresponding to $C_{20}H_{28}O_2$, one at m/z = 315.1965 corresponding to $C_{20}H_{28}O_3$, and another at m/z = 317.2122 corresponding to $C_{20}H_{30}O_3$. Table 3 gives the sum of GC-MS peak areas for the major classes of constituents in the ethanolic extracts. Diterpenoids were 9.3-fold more concentrated in L samples than in S samples; organic acids were 2-fold and phenols were 3-fold less concentrated in L samples than in S samples.

Table 2. Sum of UHPLC-HRMS peak areas for major classes of constituents in macerates prepared with leaves collected from trees exposed to solar light (L-D2, L-D4, and L-D8) and leaves collected in trees facing north (S-D2, S-D4, and S-D8) after 2, 4, and 8 days of maceration. $Area_{L\text{-}D4}/Area_{S\text{-}D4}$ represents the ratio of peak areas for L-D4 over S-D4.

Macerate	Organic Acids	Phenols	Sugars
L-D2	6.2×10^9	2.0×10^9	5.2×10^8
L-D4	14×10^9	2.3×10^9	1.9×10^9
L-D8	28×10^9	10×10^9	3.0×10^9
S-D2	3.0×10^9	4.1×10^9	3.8×10^8
S-D4	5.5×10^9	3.2×10^9	8.7×10^8
S-D8	15×10^9	5.3×10^9	1.3×10^9
$Area_{L\text{-}D4}/Area_{S\text{-}D4}$	2.5	0.71	2.2

Table 3. Sum of GC-MS peak areas for major classes of constituents in ethanolic extracts of leaves collected in trees exposed to solar light (L) and trees facing north (S) and peak area ratios.

Analysis Technique	Sample	Organic Acids	Phenols	Terpenes	Terpenoids	Diterpenoids
UHPLC	L	2.4×10^9	1.1×10^8			2.9×10^9
	S	1.2×10^9	2.9×10^8			2.8×10^8
	$Area_L/Area_S$	2.0	0.37			9.3
GC	L			1.4×10^7	2.7×10^5	2.1×10^7
	S			4.5×10^6	1.5×10^5	3.4×10^6
	$Area_L/Area_S$			3.0	1.8	6.2

The results of GC-MS analysis are shown in Figure 5. In extract L, the main components were four diterpenoids, a naphthalene derivative, D-limonene, and α-pinene; the four diterpenoids were agathadiol, communic acid, kaur-16-en-18-al, and cis-totarol. In extract S, agathadiol and kaur-16-en-18-al, the naphthalene derivative, 3-carene, sabinene, and α-pinene were the main components. Agathadiol and communic acid are labdane diterpenoids with two rings, while cis-totarol is a phenol diterpenoid with three rings and kaur-16-en-18-al a kaurane diterpenoid with four rings. The two latter components contain the decaline ring as labdane and can be considered as labdane-related diterpenoids [27]. Then the proportion of each component in the extracts was calculated in percentage and scaled between 0 and 100. This presentation of the data shows that there were higher proportions of communic acid, kaur-16-en-18-al, cis-totarol, and D-limonene in extract L than extract S, and higher proportions of 3-carene, compound X, and manoyl oxide in S than L (Figure S3).

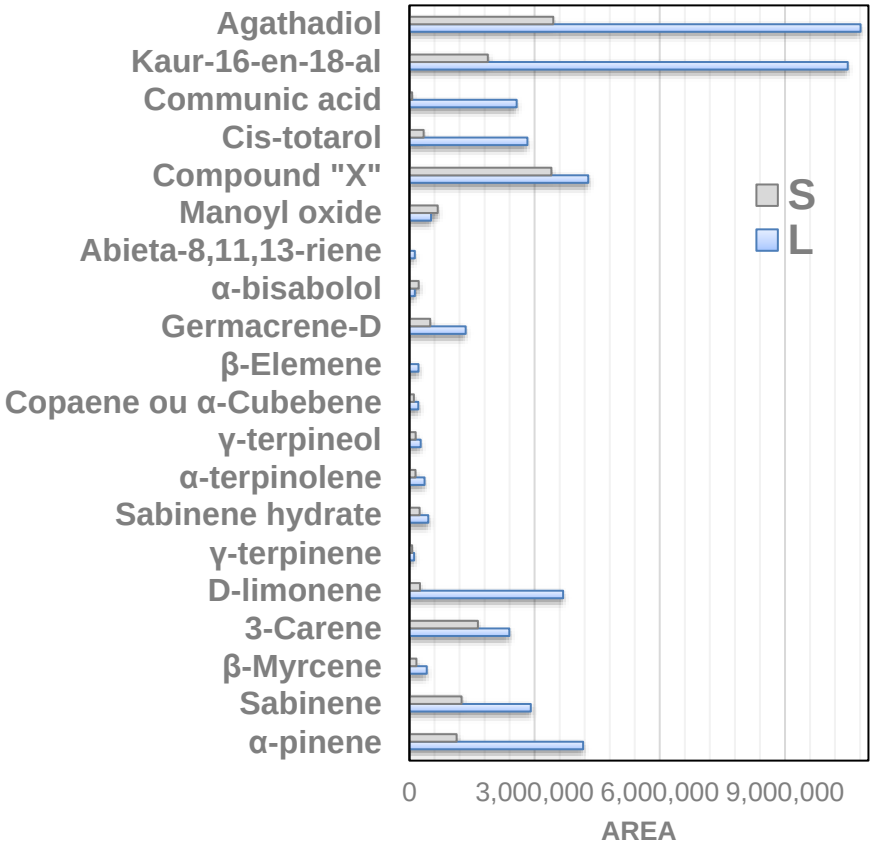


Figure 5. Main constituents of ethanolic extracts of leaves collected in trees exposed to solar light (L) and trees facing north (S). Compound "X" is (E)-3-methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl -2-methylene-deca-hydro-naphthalen-1-yl)pent-2-en-1-ol.

Because the detected labdane derivatives were not commercially available, we tested the effect of (E)Labd-13-ene-8,15-diol, which belongs to the same family of molecules. At 10^{-3} M, this compound did not show any effect on the germination rate of *Lepidium sativum*.

4. Discussion

Several reports described cypress phytotoxicity but none of them identified the molecules involved. The diversity of chemicals found in macerates and leaves makes the identification of phytototoxic compounds challenging. Moreover, the inhibiting effects of macerates and mulches might not involve the same components. Indeed, the phytotoxic effect of macerates was due to water-soluble compounds or their degradation products, while that of mulches was due to molecules reaching the soil surface by volatilization or direct contact of leaves with moistened soil.

Aqueous extracts of *Cupressus sempervirens*, a species of cypress common in Mediterranean countries, were found to show phytotoxicity against tomato and lettuce seeds, but aqueous extract analyses were not performed [21]. Duryea et al. tested water extracts of fresh mulches, including *Taxodium distichum* cypress mulch, on lettuce seeds [17]. While enriched in phenols compared to the other mulch water extracts, the cypress mulch water extract was less phytotoxic. These results did not allow the authors to draw clear conclusions regarding the contribution of phenolic compounds to the phytotoxicity of mulch water extract. Here, based on the increased phytotoxicity with maceration time, we planned to use the concentration criteria to identify phytotoxic components. However, the UHPLC-MS peak area of almost all detected components increased with maceration time and measured inhibition percentage. This common concentration evolution of all components makes the identification of potential candidates difficult.

The analyses conducted in this work revealed the presence of organic acids, phenols, monoterpenes, monoterpenoids, and diterpenoids in ethanolic extracts of *Cupressus leylandii* leaves. Volatile compounds (monoterpenes and monoterpenoids) can reach the soil by volatilization or through transfer into water, while semi-volatiles (phenols and diterpenoids) are most likely transferred via water or direct contact of leaves with soil. Monoterpenes and particularly monoterpenoids, their oxygenated counterparts, are generally identified as causing phytotoxic effects [4,5,7]; however, volatilization could reduce their efficiency, as it tends to lower their local concentration over time. When comparing data of L and S extracts (Table 3), it appears that the overall concentration of all components except phenols is higher in L extracts, which might explain their higher phytotoxicity. On the other hand, LC-MS and GC-MS analyses of ethanolic extracts revealed that diterpenoids are relatively major components of leaf extracts, particularly in L samples, which parallels its higher phototoxicity when applied as mulch. Little is known about the phytotoxicity of diterpenoids, except a few recent studies reported on kaurene diterpenoids [28–30] and labdane diterpenoids [31,32]. Unfortunately, performing experiments with these compounds is challenging due to a lack of commercial standards and/or difficulty in purifying them from natural extracts. A test with (E)Labd-13-ene-8,15-diol, which belongs to the same family, was done instead. At 10^{-3} M, this compound did not show any effect. Considering that the observed phytotoxicity is not necessarily attributed to the most concentrated components and that synergistic effects are also possible, it is clear that additional research is needed to unravel the phytotoxic effect of these mulches and the mechanism behind it.

Light is known to play an important role in plant development processes [33] and was reported to favor the production of secondary metabolites such as caffeic derivatives and flavonoids [34,35]. Interestingly, seasonal variations in the production of some terpenes were observed in cypress species, with a higher concentration of some diterpenoids in summer than in spring or winter [23], which could be also understood as an effect of light. In our case, trees exposed to solar light showed more newly formed leaves than trees facing the north, and thus had faster growth. Exposure of trees in relation to the sun might thus explain the differences observed in terms of chemical composition between leaves collected from trees exposed to solar light and trees facing the north.

5. Conclusions

To conclude, we demonstrated that *Cupressus leylandii* can help to control weeds when their leaves are used as macerate or mulch. All the chemicals detected in macerates and ethanolic extracts of leaves are potential candidates in mixtures or isolated form, and further research is still needed to firmly identify the phytotoxic components. These trees are appreciated as hedges in private gardens in European countries, and the cutting wastes could be used by gardeners. The large-scale use of green wastes from *Cupressus leylandii* brings up the question of sourcing and would probably require extensive planting operations of these woody plant species.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/11/1177/s1, Figure S1: UHPLC-HRMS chromatograms of (A) L-D4 and (B) S-D4 macerates, Figure S2: UHPLC-HRMS chromatograms of ethanolic extract of L macerate, Figure S3: Main components of ethanolic extracts of S and L leaves after normalization to 100. Table S1: Effect of macerates on seed germination. *p*-values obtained by comparing the rates of germination two-by-two using Tukey's test, Table S2: Effect on seed germination of S (shade) and L (light) macerates prepared with leaves collected in March. R is the germination rate and G_T is the total amount of germinated seeds after 7 and 5 d post imbibition. Mean values of four replicates (±standard error), Table S3: UHPLC-HRMS data of main macerate constituents. Proposed structures and ratio of peak area between L-D4 and S-D4, Table S4: UHPLC-HRMS data of main constituents of leaf ethanolic extracts and ratio of area.

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