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INDEX

- 9-14 O. Oueslati and M. Ben-Hammouda**
A laboratory approach to investigate auto-toxicity of soils cultivated with barley
- 15-21 Manuel J. Reigosa and Alejo Carballeira Ocaña**
Phytotoxicity in Topsoils Collected under *Acacia dealbata* Link in Galicia (NW Spain)
- 23-33 Manuel J. Reigosa and Alejo Carballeira Ocaña**
Studies on the Allelopathic Potential of *Acacia dealbata* Link.: Allelopathic Potential Produced During Laboratory Decomposition of Plant Residues Incorporated Into Soil





A laboratory approach to investigate auto-toxicity of soils cultivated with barley

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ABSTRACT

Monoculture of barley, where direct drilling is applied as mean of conservation agriculture, may encounter allelopathic risk. Both tissue leachates and root exudates of a crop could persist in the soil with a high depressive potential for the subsequent one. Most studies on barley allelopathy were on above-ground residues and little is known about the allelochemicals released in to the soil by living residues. Auto-toxicity of extracts from soils cultivated with 4 barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane') was studied using seedling growth bioassays, with 'Manel' as the test-variety. To investigate the role of phenolics in barley auto-toxicity, inhibition of radicle growth by soil extracts was regressed on total-phenolics and 5 phenolic acids (p-hydroxybenzoic, ferulic, vanillic, syringic, p-coumaric) contents of all soil extracts. Only radicle growth bioassays permitted the detection of barley auto-toxicity expressed by soil extracts. Radicle growth inhibition by soil extracts from a non-cultivated soil (soil-Control) was not significant, which was not the case for extracts of cultivated ones. Such result suggests that, allelochemicals were essentially released by plant tissues (leachates) and root exudates into the rhizosphere. No significant correlation was found between radicle growth inhibition by soil extracts and total-phenolic contents, though this content varied considerably among the 4 tested varieties. Three phenolic acids (vanillic, syringic, p-coumaric) were identified in soil extracts. As for total-phenolic, no significant correlation was found between radicle growth inhibition by soil extracts and phenolic acids contents. This is an indication that other allelochemicals highly determine barley auto-toxicity.

Keywords: barley varieties, soil extracts, bioassays, phenolic acids, total-phenolics.

Introduction

Understanding soil-residues interaction in the expression of an allelopathic potential could be an aid to manage allelopathic risk in case of Direct Drilling (DD). The DD is one of the conservation agriculture three principles: i) no soil disturbance and DD, ii) permanent mulching, and iii) adapted crop rotation that should be applied simultaneously. Soil extracts from DD wheat plots, revealed a significant production of allelochemicals after harvest and during the following cropping season when compared to soil extracts from conventional drilled wheat plots (Cast *et al.*, 1990). Two allelopathic rice (*Oryza sativa* L.) varieties released momilactone B into the soil, at phyto-toxic levels (Kong *et al.*, 2006). Four phenolic acids (p-hydroxybenzoic, vanillic, p-coumaric, ferulic) were identified at various levels in four different soils inducing differential plant growth (Whitehead, 1964). Working with alfalfa (*Medicago sativa* L.), auto-toxic factors were identified in sandy and clay soils, with higher inhibitory activity to seedling growth in sandy soils (Jennings

and Nelson, 1978). Coumarin and cis-cinnamoyl glucosides are two allelochemicals found in three different (volcanic ash, alluvial, calcareous) soils (Hiradate *et al.*, 2010). Decaying rice residues released five phenolic acids (p-hydroxybenzoic, p-coumaric, ferulic, vanillic, syringic) into the soil, that inhibited successive growing plants of a paddy weed, barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv.] (Kong *et al.*, 2006).

Exuded allelopathic compounds from living roots helps wheat seedlings to compete against annual ryegrass (*Lolium rigidum* Gaud.) growth (Wu *et al.*, 2001b). At different growth stages, wild oat (*Avena fatua* L.) exuded phenolic compounds into the rhizosphere, which are potentially allelopathic substances (Iannucci *et al.*, 2012). Wheat seedlings, from accessions with low allelopathic potential, did not exude detectable amounts of allelochemicals into the growth medium in comparison with strongly allelopathic ones (Wu *et al.*, 2001a, b). Jasmonic acid is an allelochemical identified in root exudates of bread wheat (Dathe *et al.*, 1994). Grain-sorghum roots [*Sorghum bicolor* (L.) Moench.], exuded 3 phenolic

acids (p-hydroxybenzoic, vanillic, syringic) that may enhance the overall allelopathic potential of sorghum in the field (Ben-Hammouda *et al.*, 1995b).

Since root exudates and residue leachates may have synergic effect in the expression of barley auto-toxicity, in a continuous cropping system, the study of this potential risk of soil extracts from soil cultivated with barley could be of great interest to cereal producers. Previous work on above-ground tissues of the same barley varieties was conducted and showed that phenolics play a role in barley allelopathy/auto-toxicity (Oueslati *et al.*, 2009). So, investigating the contribution of phenolics in barley auto-toxicity, expressed by leachates/root-exudates, would be an integrative approach to better understand barley auto-toxicity and improve cereal residues management in Tunisia under conservation agriculture cropping systems.

The present work aims to: i) investigate auto-toxic potential of soils cultivated with barley, ii) characterize the role of total-phenolics (TP) content and 5 individual phenolic acids (PA) [p-hydroxybenzoic (POH), vanillic (VAN), syringic (SYR), p-coumaric (PCO), ferulic (FER)] in the expression of such potential.

Materials and Methods

Preparation of soil extracts

Immediately after harvest, at late June 2002 soil samples were randomly collected from plots cultivated with 4 barley varieties ('Manel', 'Martin', 'Espérance', 'Rihane'), grown at the Experimental Station of Ecole Supérieure d'Agriculture du Kef. The station was located in the semi-arid zone of North-West of Tunisia on an alkaline (pH = 8.1) clay (59% clay, 23% silt, 18% sand) soil according to (Donahue *et al.*, 1983) with 2 % organic matter and classified as calcisol (Deckers *et al.*, 1983). Samples were collected across 30 cm depth; air dried during 1 day, and then passed through a sieve of 0.3 mm stitches. Soil extraction was done following the procedure described by (Read and Jensen, 1989). A sample of 250 g equivalent of dry soil was extracted in 250 ml distilled water on a horizontal shaker for 24 h at 200 rpmn. Soil suspensions were filtered across Whatman N° 2 filter paper under vacuum then stored at 5°C until bioassay. Prior to TP analysis, soil extracts were centrifuged at 12500 rpmn for 20 mn at 8 °C.

Seedlings bioassay

A 1.2 % growth medium of agar, amended with 20 ml extracts of soil cultivated with 4 barley varieties

('Manel', 'Martin', 'Espérance', 'Rihane'), was used to be tested on seedlings (coleoptile, radicle) growth of 'Manel', chosen to be a test-variety. In addition, to a control of distilled water-agar (1.2 g agar/100 ml of distilled water), a second water-agar control of non-cultivated soil (fallow and hand weeded) was considered. The second control was a mix of 1.2 g agar in 80-ml of distilled water and 20 ml water-extract from the non-cultivated soil.

Surface sterile seeds were pre-germinated in Petri dish [(180x10) mm] between 2 filter papers moistened with 1.2 ml of distilled water. Seedling bioassays were conducted, using a test tube technique. Test tubes [(180x10) mm] were covered with cotton, slanted at 45° allowing 15 ml growth medium to solidify. Seedlings with radicle 3 mm long were transplanted into tubes. After 60-h of incubation, in dark at 25°C, lengths of coleoptile and central radicle of each barley seedling were measured and recorded, a procedure reported by (Ben-Hammouda *et al.*, 2001, 2002). Radicle and coleoptile's growth inhibition [Inhibition (%) = (Control – Treatment)/control × 100] of 'Manel' by soil extracts were calculated for all bioassays.

Determination of total-phenolics

For TP analysis, Folin-Denis method was applied (1), with tannic acid (TA) as a standard. Folin-Denis reagent is a mix of 10 g of sodium tungstate, 2 g of phosphomolybdic acid and 5 ml of phosphoric acid added to 75 ml of distilled water. The mixture was refluxed for 2 h, cooled, and then diluted to 100 ml with distilled water. A saturated sodium carbonate solution was prepared by dissolving 40 g of sodium carbonate to 150 ml of distilled water for 1 h in dark, and then adjusted to 200 ml. TP content determination was made following (Makkar, 2000) procedure: Saturated TA solution was prepared by dissolving 50 mg of TA in 100 ml distilled water. Aliquots [(0, 20, 40, 60, 80, 100) µl] of standard TA solutions were dispensed into tubes containing each 0.5 ml of Folin-Dennis reagent and 2.5 ml of saturated sodium carbonate solution. Standards were diluted to 4 ml with distilled water and quickly shaken. Absorbance was determined after 35 min in dark at 750 nm by a spectrophotometer (A.O.A.C., 1990), and then a standard curve was plotted.

For soil extracts, determination of TP was realized by adding 0.5 ml of Folin-Denis reagent and 2.5 ml of a saturated sodium carbonate solution to 1 ml of extract. Absorbance was registered and TP content of each sample was estimated based on a standard curve. TP were directly expressed in µg TA equivalents/g-soil, based on an extraction ratio of 1:1 (w/w).

Determination of phenolic acids

Soil extracts of barley were also, analyzed for 5 PA (POH, VAN, SYR, PCO, FER), which are known to be associated to barley (Oueslati *et al.*, 2009) and wheat allelopathy (Wu *et al.*, 2001c). First, crude soil extracts were filtered through a 0.45 μ m sterile membrane, than analyzed for PA using a Shin-pack CLC (M-ODS) HPLC system. The system consists of: i) 2 pumps operating at 0.7 ml/min flow rate, and ii) a wavelength UV-detector, set at 280 nm. Separation of PA was done on a C18 reverse-phase. The mobile phase consisted of 0.1% phosphoric acid in 70% acetonitrile. Quantification of the individual PA was managed by a computerized package.

Data analysis

The experimental design for seedlings (radicle, coleoptile) growth bioassays was a CRD with 4 repetitions. Bioassays data were submitted to an analysis of variance, using SAS package (SAS Institute, 1985) and Fisher's protected test for least significant difference (LSD) at the 0.05 level of probability (Steel and Torrie, 1980), to make mean separation. A correlation analysis between the inhibitory effects of soil extracts on barley radicle growth and TP contents was conducted using SAS package. A second correlation between the inhibitory effects of the same extracts on barley radicle growth and PA contents was also conducted.

Results and Discussion

Extracts from soils cultivated with 4 barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane')

and from a non-cultivated soil (soil-control) had no significant effects on 'Manel' coleoptile's growth. Though, all soil-extracts had a significant effect on radicle growth, excepting soil-control. As expected, radicle growth bioassays were the most reliable test to detect auto-toxic potential of soil extracts cultivated with barley. Similar results were obtained by (10) and (Ben-Hammouda *et al.*, 2001, 2002), working on oat (*Avena sativa* L.) and barley allelopathy, respectively. Soil-control expressed a slight but not significant (15 %) inhibition when compared to water-control. This shows that a non-cultivated soil could release phytotoxic substances in addition to those potentially leached by above-ground biomass and exuded by roots. A kind of activity that could be attributed to soil microbial effect (Waniska *et al.*, 1988; Sturz and Christie, 1996; Ito *et al.*, 1998; Kong *et al.*, 2007).

Soil extracts that are relevant to 'Esperance' were the most inhibitory, reducing radicle growth by 29 % and 39 % when compared to soil-control and water-control, respectively. 'Manel' soil extracts were the least inhibitory, reducing radicle growth only by 17 % and 29 % when compared to soil-control and water-control, respectively. The later and the former results indicate that the varietal auto-toxicity is less pronounced than the varietal hetero-toxicity. Using an increasing order of inhibition, tested varieties were classified as follows: 'Espérance' > 'Martin' > 'Rihane' > 'Manel' (Table 1). The differential inhibitory effect of soil extracts, among barley varieties, is always higher when compared to soil-control. Such differences could be due largely to leachates/root-exudates of barley. These results are in agreement with those found with grain-sorghum (Ben-Hammouda *et al.*, 1995a) and rice allelopathy (Om *et al.*, 2002).

Table 1.

Effects of extracts from cultivated soils with 4 barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane') on radicle growth (cm) of 'Manel', during the 01/02 growing season.

Treatment	Radicle growth
Water-control	5.30 a*
Soil-control	4.52 ab
Soil extract/'Manel'	3.75 bc
Soil extract/'Rihane'	3.28 c
Soil extract/'Martin'	3.25 c
Soil extract/'Espérance'	3.23 c
LSD (5%)	0.97

* Means followed by the same letter are not significantly different at 5% level of probability.

There were great differences in TP content among soils cultivated with the four barley varieties. Phenolics were not detected in soil extracts relevant to 2 varieties ('Manel', 'Martin'), while those of 'Rihane' had the highest TP content. Similar results were found about differences of phenolics in soil cultivated with three sorghum hybrids (Ben-Hammouda *et al.*, 1995b). The relatively high amount of TP (1.27 μg /g-soil), in soil-control (Table 2) could be explained by decaying old residues (more than one growing season) or by microbial activity products (Whitehead *et al.*, 1981).

Correlation analysis between inhibition of 'Manel' radicle growth by barley soil extracts and their relative TP contents showed no significant association between the two parameters. Such result, suggests that barley auto-toxicity is not highly determined by phenolics. The fact that 'Martin' with no detected phenolics had a higher inhibitory activity than

'Rihane' with 3.89 μg /g-soil as a TP content explains clearly the absence of a cause-effect relationship.

Soil extracts analysis revealed that 3 (VAN, SYR, PCO) out of 5 (POH, VAN, SYR, PCO, FER) targeted PA were present with low concentrations, varying slightly among varieties. While in a precedent work realized on tissues of the same barley varieties the five targeted PA, were generally present (Oueslati *et al.*, 2009). PCO was detected only in extracts of soils cultivated with 'Manel' and 'Esperance', but SYR was the only PA found in all extracts. Its concentration was higher in soil extracts when compared to its concentration in soil-control, by about 2, 3, 6, and 10 times, respectively for soil extracts of 'Rihane', 'Manel', 'Esperance' and 'Martin'. This suggests that SYR could be produced by leachates/root-exudates and soil microbial activity, but with less intensity by soils (Table 2).

Correlation analysis, between inhibition of radicle growth of barley by soil extracts and TP

Table 2.
Total phenolic (TP) and phenolic acid (PA) contents ($\mu\text{g/g}$ -soil) of extracts from cultivated soils with 4 barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane'), during the 01/02 rowing season.

Source of extracts	TP Content	PA*	PA Content
Soil-control	1.27	VAN	0.002
		SYR	0.001
		PCO	0.000
Soil extract/'Manel'	0.00	VAN	0.001
		SYR	0.003
		PCO	0.001
Soil extract/'Martin'	0.00	VAN	0.002
		SYR	0.010
		PCO	0.000
Soil extract/'Esperance'	0.20	VAN	0.000
		SYR	0.006
		PCO	0.001
Soil extract/'Rihane'	3.89	VAN	0.001
		SYR	0.002
		PCO	0.000

*PA Vanillic (VAN), syringic (SYR) and p-coumaric (PCO).

content of these extracts, revealed no significant association. Also no association, between inhibition of radicle growth and PA contents, was found. Three of the targeted PA (POH, SYR, PCO) were identified responsible of the auto-toxicity of tissues from the same barley varieties (Oueslati *et al.*, 2009). Such result suggests that barley auto-toxicity appeared to be more determined by growing season and other types of allelochemicals, such the 2 protoalkaloids (hordenine, gramine) found in tissues extracts and root exudates of barley (Liu and Lovett, 1993a, 1993b). The probability of indirect effect of phenolic compounds should not be omitted. A future work with an experiment separating leachates from root exudates will better express their relative intensity.

Conclusion

Auto-toxic potential of barley was identified in soil extracts, using the technique of seedlings growth bioassays. Such potential varied among soils where barley varieties were cultivated. Neither TP content nor individual PA concentrations of soil extracts were able to explain barley auto-toxicity. The possibility of implication of other allelochemicals than phenolics should be taken in consideration. Leachates/root-exudates must be considered in any case study of barley allelopathy to assess its over-all potential, before making decision at the farm level to go for a barley/barley sequence especially in a conservation agriculture cropping system based on a permanent mulching and a direct drilling . A kind of decision that becomes more delicate when direct drilling is practiced, as a mean to rebuilt soil fertility and control erosion.

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Phytotoxicity in Topsoils Collected under *Acacia dealbata* Link in Galicia (NW Spain)

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ABSTRACT

Topsoils were collected 41 times along one year under the vegetal cover of *Acacia dealbata*, and their phytotoxicity was assessed using *Lactuca sativa* var. Great Lakes bioassays.

The results show that toxicity is not so pronounced as when other kinds of assays are carried out (decomposition of vegetal residues in the laboratory, artificial and natural washes) so being confirmed that the soil can act either by soaking up or by inactivating the allelopathic agents by means of microbial action. The maximum toxicity appears after that flowers have fallen down on the soil and their decomposition has begun what means that decomposition of vegetal residues is probably the most important release way of allelochemicals. The importance of the phenological gap between the blooming of *A. dealbata*, exotic species and the germination periods of the autochthonous herbaceous species is discussed.

Keywords: Allelopathy, *Acacia dealbata* Link, *Lactuca sativa* L., Soil Toxicity.

Introduction

The genus *Acacia* includes some species that are invasive in Europe and other parts of the world (Lorenzo *et al.*, 2010). Two of the most invasive species are *A. melanoxylon* and *A. dealbata*, have been blamed to release some phytotoxic compounds (Hussain *et al.*, 2011a, 2011b, González *et al.*, 1995, Reigosa *et al.*, 1995). Although there are some controversial results about the role of allelopathy in their invasive capacity (Lorenzo *et al.*, 2008, 2010), it is clear that the ecosystems are affected by *A. dealbata*, both soils (Souza *et al.*, 2014), microorganisms (Lorenzo *et al.*, 2013) and the understorey once the plants are established (Lorenzo *et al.*, 2011, 2012). Effects produced by allelochemicals in the invasion process are difficult to assess, and they can be direct or indirect (Belz *et al.*, 2009; Grove *et al.*, 2012), including the effects on pathogens (Zhang *et al.*, 2009). And the effects can be mediated by the soil characteristics, including the nutrients (Xiao *et al.*, 2007). After establishment, the high production and release of allelochemicals could even produce autotoxic effects (Politycka, 2005, Zhang *et al.*, 2001).

The soils are the main means of transmission of allelopathic agents, so it is very interesting the study of their effect on germination and growing

of plants. It is in the soil where vegetal residues gather, which are decomposed by biotic or nonbiotic factors and in the decomposition compounds having allelopathic capacity can be released, either directly or by subsequent degradation (Kaminsky, 1981; Molina *et al.*, 1991; Whitehead, 1964). Allelopathic substances coming from aerial parts leaching or from radicles exudation can appear on the soil (Patrick, 1971; Robinson, 1972) and even compounds volatilized to the environment can appear fixed on the soil after being swept down by dew (Del Moral & Muller, 1969; Rice, 1974, 1984).

Still more information is needed on the production, accumulation, persistence and stability of allelopathic substances in the soil (Inderjit & Bhowmik, 2004, Reigosa *et al.*, 1999a, Rice, 1979, 1984, Zhang, 1993) but it has been verified the influence of different environmental factors (Guenzi & Mac Calla, 1966; Koeppe *et al.*, 1970a, 1970b, 1976; Muller & Chou, 1972; Patrick & Koch, 1958; Stevenson, 1967) as well as the action of fungi and bacteria (Gant & Clebsch, 1975; Grant, 1976; Jalal & Read, 1983; Kaminsky, 1981; Kung, 1971; Leon, 1976; Tack *et al.*, 1972; Turner & Rice 1975; Vaughan *et al.*, 1983). Substances with allelopathic capacity have a very short average life in the soil specially phenols, even though some substances (like

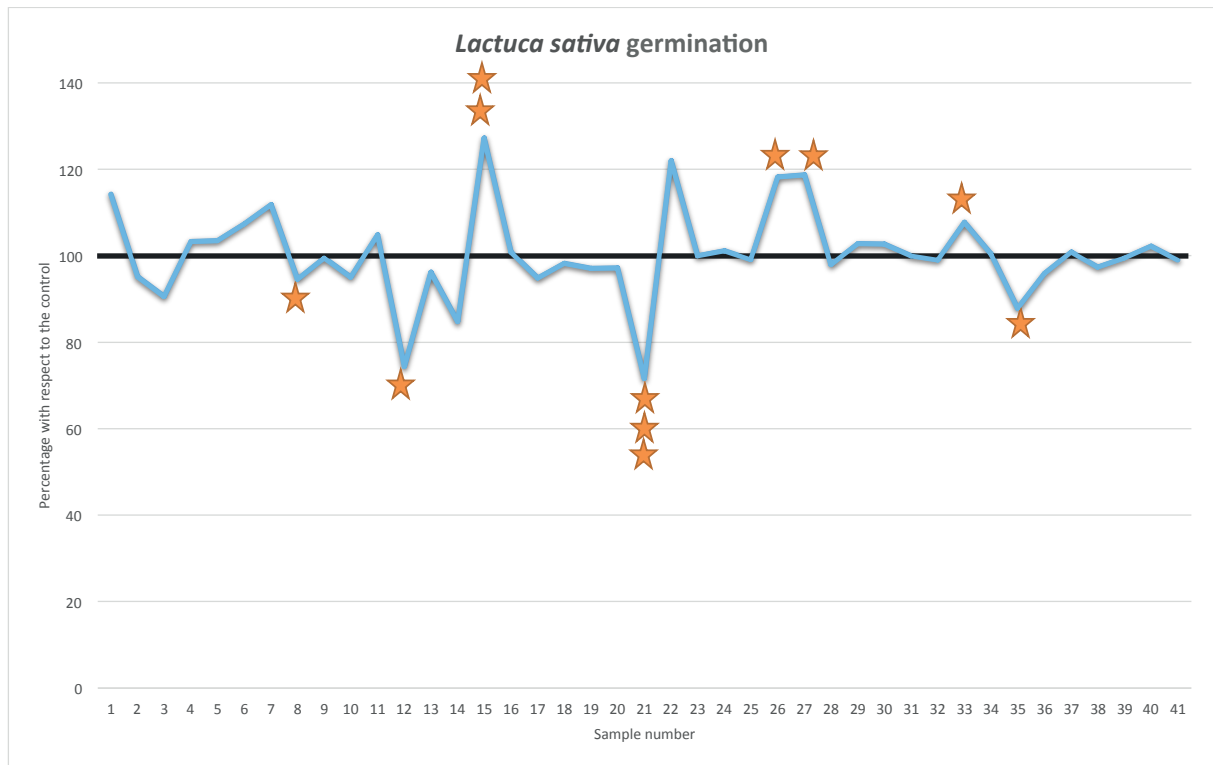


Figure 1.

Germination of *Lactuca sativa* in topsoils compared to control. Results are expressed in percentage with respect to the control. Statistical differences are shown as follows: * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

condensed tannins) take a long time to be degraded (Grant, 1976; Jalal *et al.*, 1982; Jameson, 1970; Jose and Gillespie, 1998, Levitt *et al.*, Shindo & Kuwatsuka, 1977; Wang *et al.*, 1971). It has been demonstrated (Reigosa *et al.*, 1984, 1985) the presence of phenols as hydroxibenzoic, protocatechuic, gentisic, p-cumaric, and ferulic acids in soils under the *Acacia dealbata*. Once released, allelochemicals can affect several physiological processes, including germination and growth (Chiapusio *et al.*, 1997, Reigosa and González, 2006, Reigosa *et al.*, 1999b)

The works aimed at clarifying the temporal evolution of phytotoxicity are not very large either (Carballeira & Cuervo, 1980; Casal *et al.*, 1985; Lodhi, 1975, 1976; MacPherson & Muller, 1969; Reigosa *et al.*, 1984, 1991, 2016; Robinson, 1971; Weidenhamer & Romeo, 1989). These diachronic studies are necessary, as the concentration of phytotoxins in the soil is very variable pursuant to the phenological cycle of the producing plant and the environmental factors, this lead to the fact that the measured values are not at all representative (Jalal & Read, 1983). Such variations can even appear in a particular phenological stage and in

periods as short as one week (Reigosa *et al.*, 1984). Additionally, there is the possibility of a temporal accumulation of toxins in short but critical periods, by considering the vital cycle stages of compounds release on the part of the plant and the washing periods (Leon, 1976; Quarterman, 1973).

So, it was designed a bioassay near to the field conditions, and it was carried out with a certain periodicity all one year round, to set up the conditions of germination all time long.

Material and methods

In a parallel way to the series of experiments by using natural and artificial leachates as described in Reigosa & Carballeira (1991, 2016) and taking into account the importance of the soil as an action place for most of phytotoxins and because of the possibility that the microbiological activity of the soil might change the allelopathic capacity of the released allelochemical agents, a series of experiments with soil collected under the arboreal cover dominated by *Acacia dealbata* Link widely described in Reigosa & Carballeira (1985) was carried out.

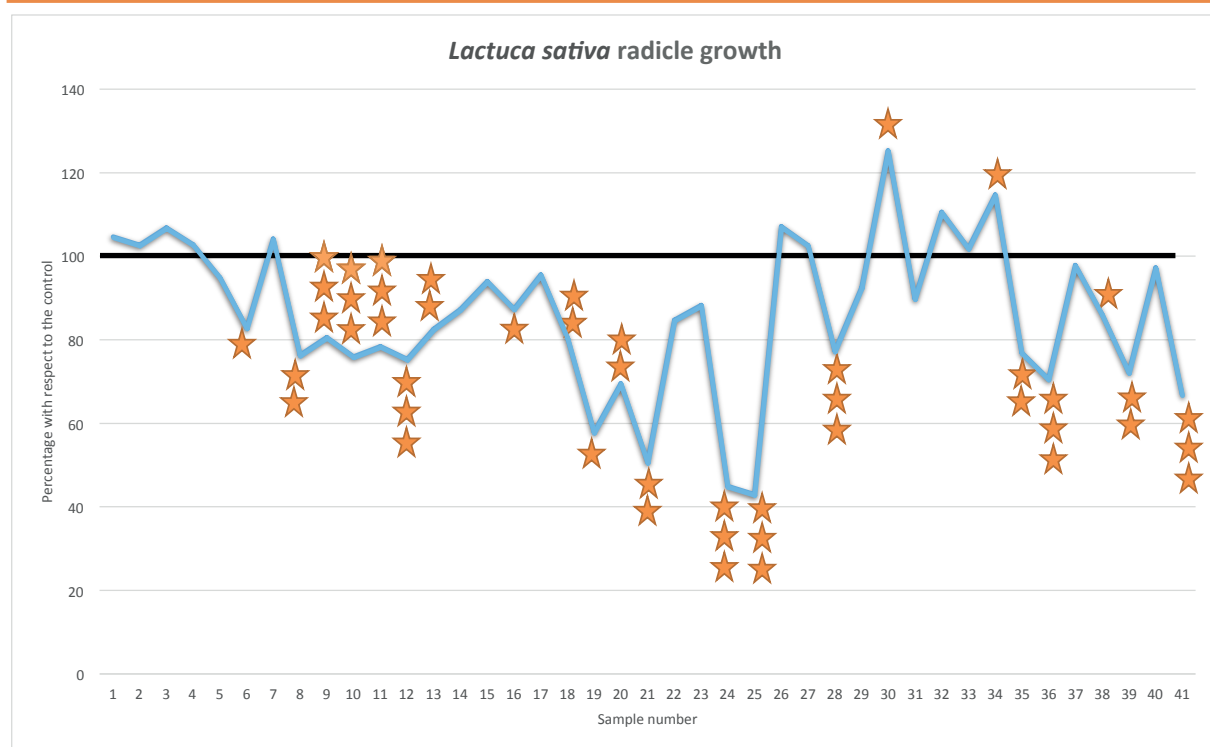


Figure 2.

Radicle growth of *Lactuca sativa* in topsoils compared to control. Results are expressed in percentage with respect to the control. Statistical differences are shown as follows: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

The used process was similar to the described in Molina *et al.* (1991). It was proceeded with the recollection of topsoil portions (the first 30 cm, considered as seeds' germination place) at five points chosen at random into a piece of ground. Once in the laboratory, all the material was homogenized and it was introduced an aliquot into each of five plates Petri, which reached a height of 0.5 cm. If the soil was at field capacity, it was only covered with Whatman 3 MM paper and it was assayed directly, by sowing 50 seeds of *Lactuca sativa* var. Great Lakes, by using as control the same number of plates with two Whatman 3 MM papers without soil and directly watered with distilled water. Should the soil be not damp enough, distilled water was added to, up to get the field capacity.

Statistical analysis. The meaning of each of assays was studied by means of oneway analysis of variance (Sokal & Rohlf, 1979). A multiple stepwise regression analysis (Afifi & Azen, 1979; Pimentel, 1979) was also carried out to research for the relationship between the allelopathic capacity of soils and different climatic factors.

Results and discussion

The results of germination bioassays are summarized in the fig. 1. It is proved that the effects are relatively limited: among the 41 assays, statistically significant differences are only found concerning the control in 7 (four stimulating and three depressing the germination). Toxicity appears during the blooming and postblooming stage.

Regarding the radicle growth (fig. 2), clear statistically significant differences are observed, so both of the stages can be more clearly marked out. An initial one of inhibition related to blooming and another one of light stimulation during the period of the year farther from the blooming. As a whole there are 20 cases of statistically significant inhibition, so prevailing the highly significant differences in front of only three cases having significant stimulation (and never overpassing the level of 5%)

This coincides with the obtained results from other assays (Reigosa *et al.*, 1984; Casal *et al.*, 1985) and confirm the flowers as an important source of active allelopathic substances. In the natural leachates and drips the inhibiting action is declared sparingly

Table 1.
Selected equation for better explanation of the effects of topsoils on germination of *Lactuca sativa*.

R multiple	0.3612				
Square R multiple	0.1305				
Square R adjusted	0.0859				
Standard Error of Estimate	17.9005				
Analysis of variance					
	Sum of squares	D.F.		Mean Square	F ratio
Regression	1875.56780		2	937.7839	2.93
Residue	12496.72500		39	320.42880	
Variables in the equation:					
Variable	Coefficient	Estándar error of coefficient	st. Reg. Coef	Tolerance	F to remove
Rain 3 days before	0.63199	0.3790	0.249	0.99997	2.78
Temperature	-1.03498	0.5937	0.260	0.99997	3.04

during the blooming while the effect in the soil is something more delayed, and it seems to coincide with a decomposition stage of flowers (Reigosa & Carballeira, 2016). The results seem to point out that the activity phytotoxins released in throughfall and stemflow (Carballeira & Reigosa, 1999) can be reduced or neutralized, what coincides with other results (Stowe, 1979).

The results of multiple regression analysis (Tables 1 and 2) show as the germination and the radicles growth in the soil assays are negatively correlated with the quantity of fallen floodwater, so it is confirmed that the flooding favors the inhibition (Rice, 1984) and positively correlated with the ionic levels in the percolates (what aimed at the same sense). Temperature also seems to show a certain importance, probably because of its influence over the decomposition rate.

The above mentioned confirms that the greatest source of effective allelopathic substances comes, sparingly, from the decomposition of vegetal residues (Ballester, 1972, Gong *et al.*, 2016, Teasdale *et al.*, 2012). It is also confirmed the prevalence of flowers as suppliers of the greatest quantity of effective allelopathic substances (Reigosa & Carballeira, 2016; Carballeira & Reigosa, 1999; Casal *et al.*, 1985). It is also proved that discordance among the phenological cycles of exotic plants (the producing ones of toxins) and autochthonous plants (receiving ones) can become fundamental in the existence of allelopathic phenomena (Rabotnov, 1974), among other reasons because the blooming of the *A. dealbata* is carried out during the germination and growing period of autochthonous herbaceous species (Reigosa *et al.*, 1984, 1999).

Table 2.
Selected equation for better explanation of the effects of topsoils on radicle growth of *Lactuca sativa*.

R multiple	0.5019				
Square R multiple	0.2519				
Square R adjusted	0.1939				
Standard Error of Estimate	20.1939				
Analysis of variance					
	Sum of squares	D.F.		Mean Square	F ratio
Regression	5217.15810		3	1739.05300	4.26
Residue	15496.10700		38		
Variables in the equation:					
Variable	Coefficient	Estándar error of coefficient	st. Reg. Coef	Tolerance	F to remove
Rain 2 days before	0.97639	0.4643	0.303	0.95014	4.42
Rain 5 days before	-0.73007	0.5142	-0.215	0.86180	2.02
Number of flowers	0.31557	0.1077	0.433	0.90434	8.59

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Studies on the Allelopathic Potential of *Acacia dealbata* Link.: Allelopathic Potential Produced During Laboratory Decomposition of Plant Residues Incorporated Into Soil

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ABSTRACT

Allelopathic effects produced during the decomposition of different plant materials coming from *Acacia dealbata* incorporated into soil were studied by means of two bioassays on *Lactuca sativa* var. Great Lakes, *Trifolium repens* and *Lolium perenne*. Results confirm their extraordinary potential allelopathic capacity. In particular, decomposing leaves showed high phytotoxic capacity followed by flowers. The third assayed material, roots, happened to be the least inhibiting one, even though at different moments it inhibited significantly the germination and the radicle growth of the assayed species.

Toxicity keeps high during long periods, even up to the end of experiences (16 weeks), with some differences related to the assayed receiving species.

Keywords: *Acacia dealbata*, *Lactuca sativa*, *Trifolium repens*, *Lolium perenne*, phytotoxicity, allelopathy, plant residues decomposition

Introduction

Allelopathy is a very widespread phenomenon in the nature (Rice, 1974, 1979, 1984; Reigosa *et al.*, 1999) but it should not be considered to have an universal character because we should expect that plants, that have co-evolutioned, will be adapted to their corresponding metabolites emission (Harper, 1977; Muller 1969; Rabotnov, 1974; Reigosa *et al.*, 1999; Teasdale *et al.*, 2012).

Allelopathic natural effects produced by tree species on understorey plants have been often found (AlMousawi & AlNaib, 1975, 1976; AlNaib & AlMousawi, 1976; AlNaib & Rice, 1971; Davis, 1928; Del Moral & Cates, 1971; Del Moral & Muller, 1969; Gliessman, 1978; Jose & Gillespie, 2006; Lee & Monsi, 1963; Lodhi, 1976, 1978; McPherson & Thompson, 1972; Mensah, 1972; Molina *et al.*, 1991; Reigosa & González, 2006; Souza-Alonso *et al.*, 2014; Tubbs, 1973).

Several species of *Acacia* genus, mainly *A. dealbata* (Casal *et al.*, 1985; Carballeira & Reigosa, 1999; Lorenzo *et al.*, 2008, 2010, 2011; Reigosa & Carballeira, 2016; Reigosa *et al.*, 1984) and *A. melanoxylon* (González *et al.*, 1995; Hussain *et al.*, 2011a, 2011b) have phytotoxic properties and have shown some potential allelopathic

phenomena, perhaps contributing to their high invasive capacity (Al Harum *et al.*, 2015a; Grove *et al.*, 2012; Lorenzo *et al.*, 2010, 2012, 2013; Zhang *et al.*, 2009).

Allelochemicals can be released to the environment by different ways: exudation of compounds through the root, leaching of aerial parts, decomposition of plant residues, release of volatile compounds and release of organic compound through seeds and fruits (Al Harum *et al.*, 2015a, 2015b; Ballester, 1971; Guo *et al.*, 2016; Reigosa *et al.*, 1999; Rice, 1974, 1984; Stowe & Kil, 1981; Teasdale *et al.*, 2012; Whittaker & Feeny, 1971; Xiao *et al.*, 2007; Zhang & Yu, 2001). The plant litter is an important component of the soil and during the decomposition process phytotoxic substances can be released or synthesized. Litter decomposition has been considered as the greatest source, in general, of effective allelopathic substances (Ballester, 1972; Bonanomi *et al.*, 2016; Chaves *et al.*, 2015; Del Moral & Cates, 1971; Guo *et al.*, 2016).

Toxicity of these substances existing in the soil depends on their nature and concentration, and on the conditions (aerobiosis or anaerobiosis, humidity, temperature) where the decomposition process is carried out, so resulting, in general, a greatest toxicity under anaerobiosis conditions; soil humidity and

temperature also affect (Al Harum *et al.*, 2015b; Jankju, M.; Patrick, 1971; Patrick & Koch, 1958; Rashid *et al.*, 2005; Ruan *et al.*, 2016; Uddin *et al.*, 2014).

On a previous study (Reigosa *et al.*, 1984) phenols with phytotoxic potential were found on the soils collected under *A. dealbata*; allelopathic potential capacity of soil extracts was also assessed (Reigosa & Carballeira, 2016). Likewise it shall be pointed out, from the mentioned study, the fast variations on such allelopathic capacity. By means of the present study it is intended to know if the different plant residues coming from the *Acacia dealbata* can lead to allelopathic phenomena when they are incorporated in the soil.

Material and methods

The area where the samples were taken has been widely described in a previous work (Carballeira & Reigosa, 1999).

The assay of plant residues decomposition in the laboratory was divided into two stages. For the first, named "Decomposition of Plant Residues I", roots and leaves of *Acacia dealbata* incorporated with an inorganic commercial balanced soil in two concentrations are used: 10% in weight or high ratio and 3% or low ratio; additionally mixed mixtures made up of roots and leaves having the same proportion both of them, and reaching the whole 10% and 3% in weight. Original commercial soil having no added plant material was used as control.

In order to prepare each mixture both the soil and the plant material was dried in sufficient amounts to cover all the assay. The plant material was ground into a ball grinder and, once it was turned into powder, it was mixed with soil taking into account the mentioned proportions. Initially the whole of treatments were macerated, all time at field capacity, into a chamber at 28° C and the material was withdrawn after 1 day, 1 week, 2 weeks, 4 weeks, 8 weeks and 16 weeks, these being used in the bioassays referred to as I.1, I.2, I.3, I.4, I.5 and I.6 from now on (Arias, 1982; Menges, 1988).

Three replications were done per treatment and they were used for the bioassay (including control) by sowing 100 seeds per Petri dish. *Lactuca sativa* var. Great Lakes was used for all the treatments and *Trifolium repens* and *Lolium perenne* only for treatments having a low ratio of plant material (roots, leaves or a mixing of both of them).

The assay we will name "Decomposition of Plant Residues II" followed a similar methodology, but with flowers and leaves and by bioassaying those portions having a high proportion of plant material with *L. perenne* and *T. repens*. This bioassay was brought under maceration as previously explained and was

withdrawn to be bioassayed after 1 day, 1, 2, 4, 8 and 16 weeks so the assays were named II.1, II.2, II.3, II.4, II.5 and II.6 respectively.

Statistical treatments. Results were tested for normality and homocedasticity. One-way ANOVAs were performed to test the existence of statistically significant differences, and then LSD tests were performed.

Results

Decomposition of Plant residues I

The results corresponding to the bioassays carried out on *Lactuca sativa* have been summarized in the fig. 1 (Germination bioassays) and in the fig. 2 (Radicle growth bioassay). Concerning the germination, there are clear high inhibiting capacities starting since the assay I.3 (corresponding to two weeks after the beginning of the experiment). On some treatments, specially, on the high concentration leaves, the inhibiting capacity is kept up to the end of the experiment. It stands out the inhibiting capacity of treatments containing leaves in front of those containing only roots. The inhibiting effect shown on radicle growth is still more pronounced than on germination. The assays showing a greater inhibiting capacity are those from I.4 to I.6 (4 to 16 maceration weeks), the same as in the germination results; it stands out in both of them the inhibiting capacities of treatments having a high concentration over those having low concentration and those including leaves.

The two other seeds (*Lolium perenne* and *Trifolium repens*) were only assayed with treatments corresponding to low concentrations, but except for little discrepancies, the results confirm the behavior of *Lactuca sativa*. Such results are summarized in the fig. 3 and 5 (Germination) and 4 and 6 (Radicle length). The greatest toxicity of leaves in front of roots is confirmed, as well as the maxima of inhibition between 4 and 16 maceration weeks. It can also be remarked that *T. repens* appears to be more inhibited than *L. perenne*.

It was also carried out a reliable test with treatments having a high concentration during the assay I.6 on both of these species and it was found as for the case of *L. sativa* a germination level of 0%.

Decomposition of Plant Residues II

The germination results of *L. sativa* are shown in the figure 7. Again the parts having a high proportion of leaves (either alone or mixed with flowers) turn out to be the most inhibiting ones, but mixtures of flowers having high or low concentration turn out to be quite inhibiting ones, especially on the assays II.3

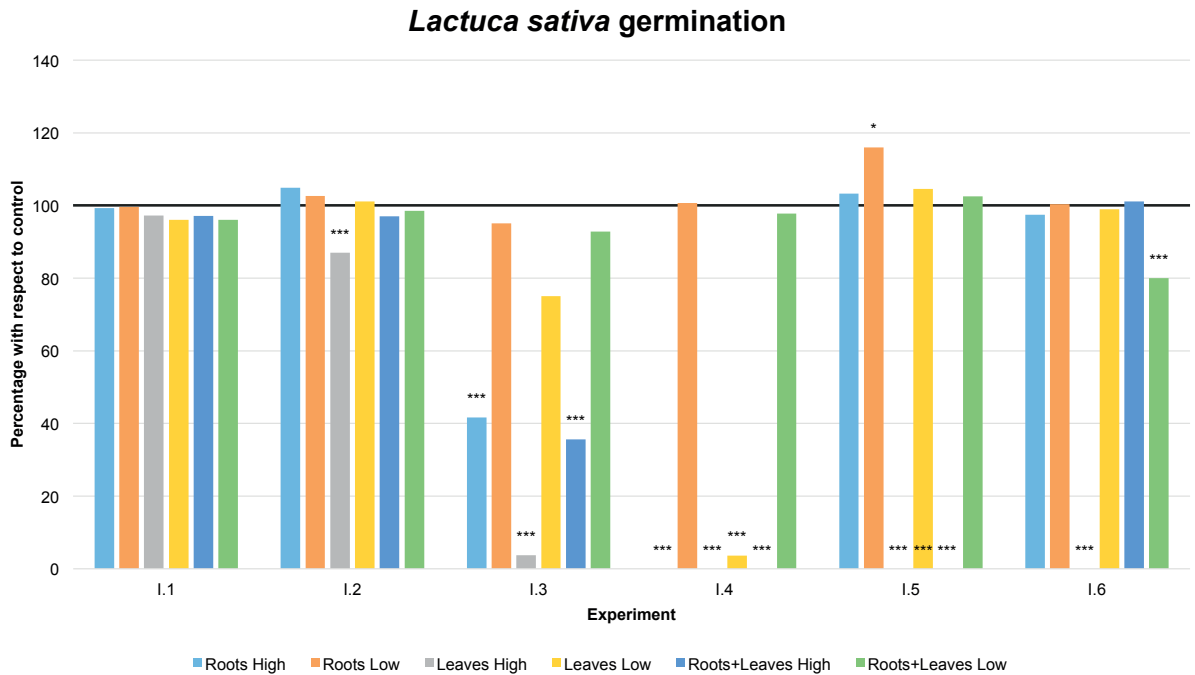


Figure 1.

Decomposition of Plant Residues I. Germination bioassays. Species Used: *Lactuca sativa*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

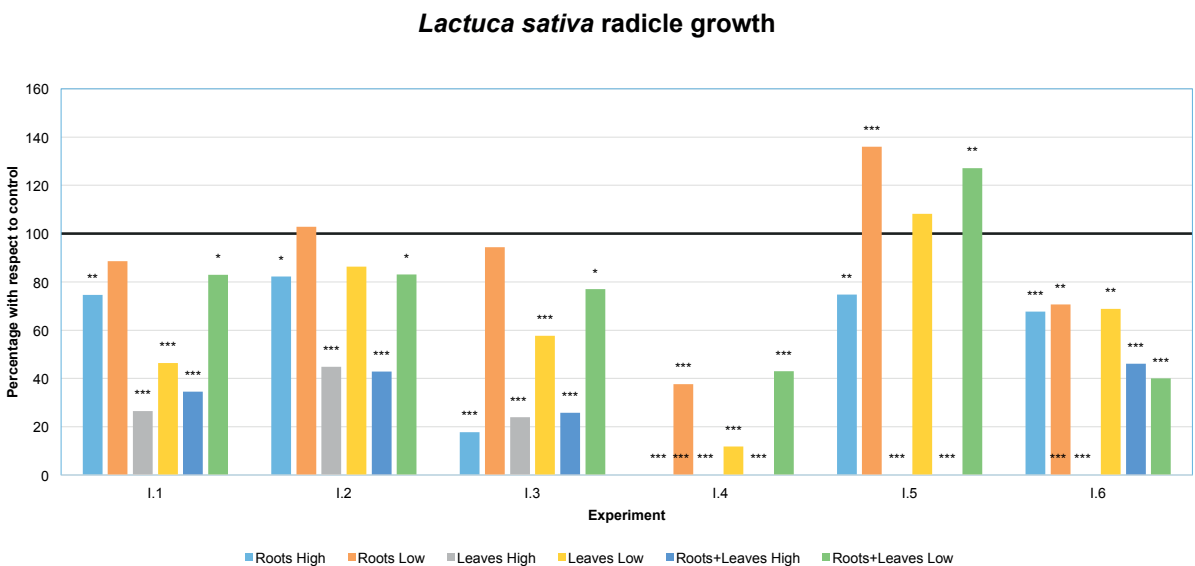


Figure 2.

Decomposition of Plant Residues I. Radicle growth bioassays. Species Used: *Lactuca sativa*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

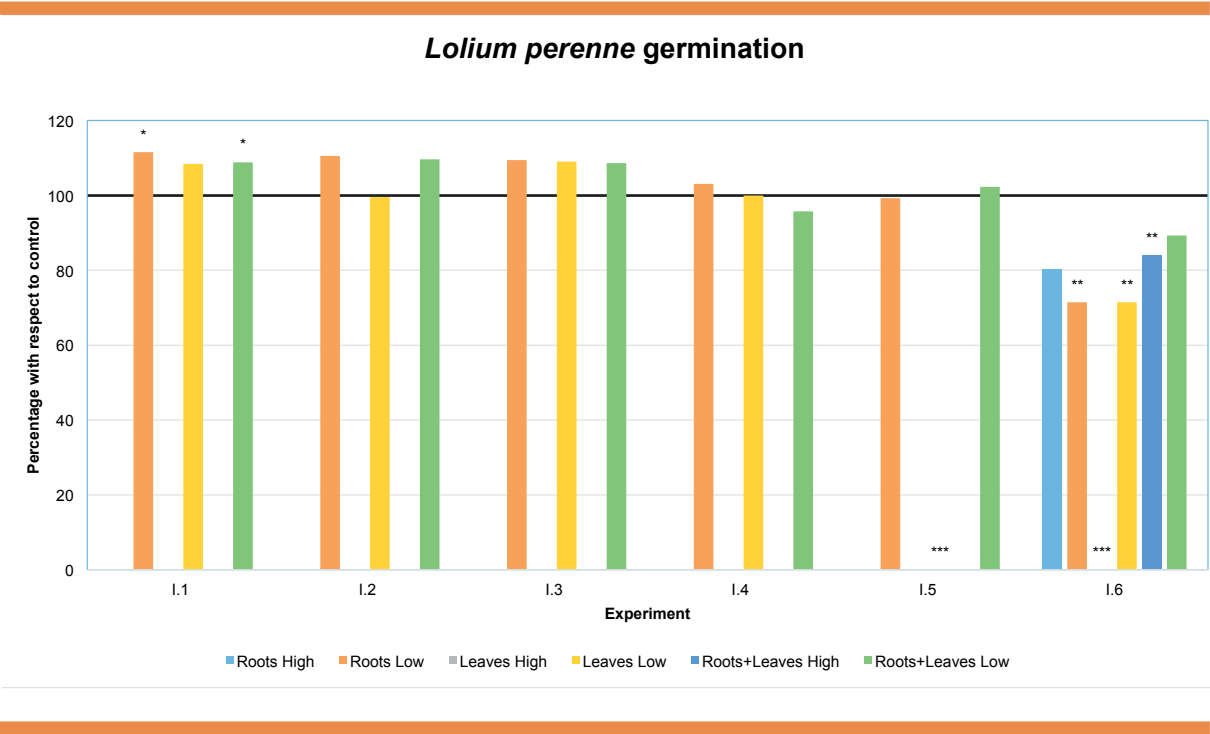


Figure 3.
Decomposition of Plant Residues I. Germination bioassays. Species used: *Lolium perenne*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

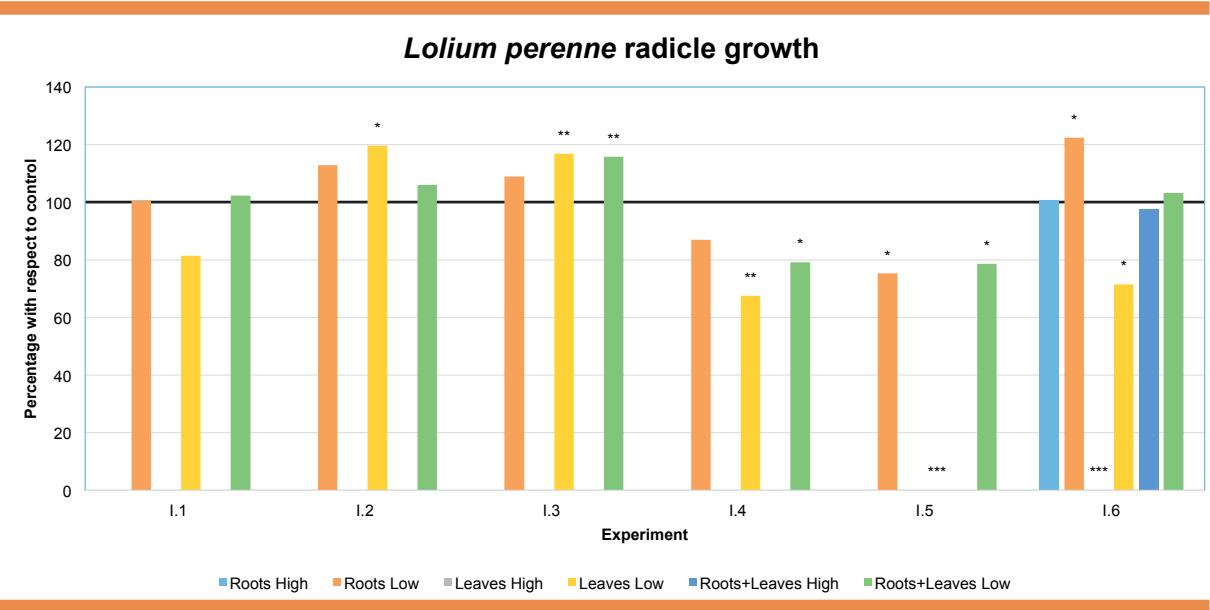


Figure 4.
Decomposition of Plant Residues I. Radicle growth bioassays. Species used: *Lolium perenne*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

and II.4 (corresponding to 2 and 4 maceration weeks), as well as the part having low concentration of leaves. At the end of the series of experiences (II.5 and II.6) strong inhibitions are kept in treatments with high proportion of leaves.

The effects on the radicles growth of *L. sativa* are shown in the fig. 8. The inhibition pattern is appreciably similar to the one shown on germination, even though it is more pronounced.

The effects on the germination of *L. perenne* and *T. pratense* are summarized in the figures 9 and 11. The effects are quite less drastic than on *L. sativa*, so statistically significant inhibition is only found on some assays of leaves in high concentration. The results corresponding to the radicles growth are shown in the figures 10 and 12. The results demonstrate slightly higher inhibiting capacity, especially on *T. repens*. The strongest inhibiting effects appear on the assays II.4 and II.5, especially on leaves in high concentration. The flowers also show some statistically significant inhibiting effect.

Discussion

The decomposition of plant residues in the laboratory demonstrates the great allelopathic potential of plant residues coming from *A. dealbata*, especially the leaves and also to some extent the flowers and, at a smaller lever the roots, during all the period that these experiences were carried out. Therefore, phytotoxicity was very high even though we will compare them to other producing species (Chiapusio *et al.*, 1997; Juste *et al.*, 1985).

The assayed proportions turn out to be very close to the field values, such as it is revealed by another work (González *et al.*, 1995) where it has been studied by the litter bags technique, the real allelopathic capacity produced *in situ* as well as the excessive foliage contribution to the soil all the year round. Therefore, we can come to the conclusion that this saprocynodynamic process of toxins release can play a very important allelopathic role, so its effect is reinforced by other eccrisadynamic processes as the leaching of aerial parts (Reigosa *et al.*, 1999).

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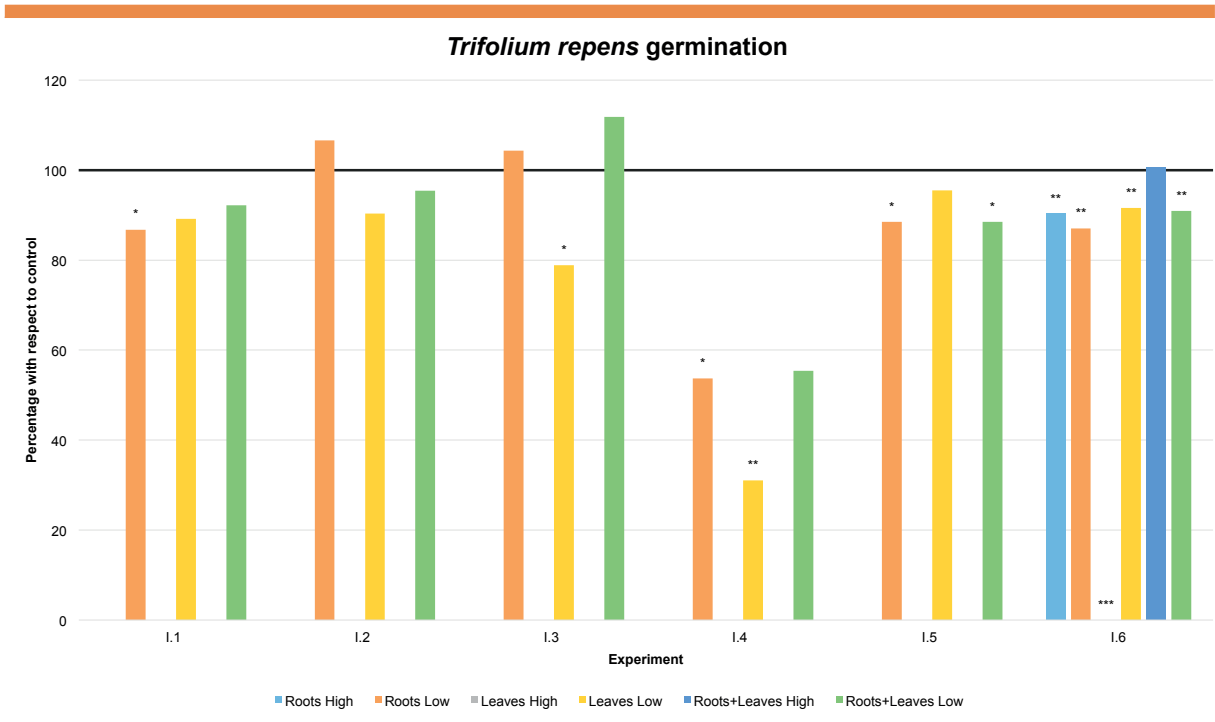


Figure 5.
Decomposition of Plant Residues I. Germination bioassays. Species used: *Trifolium repens*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

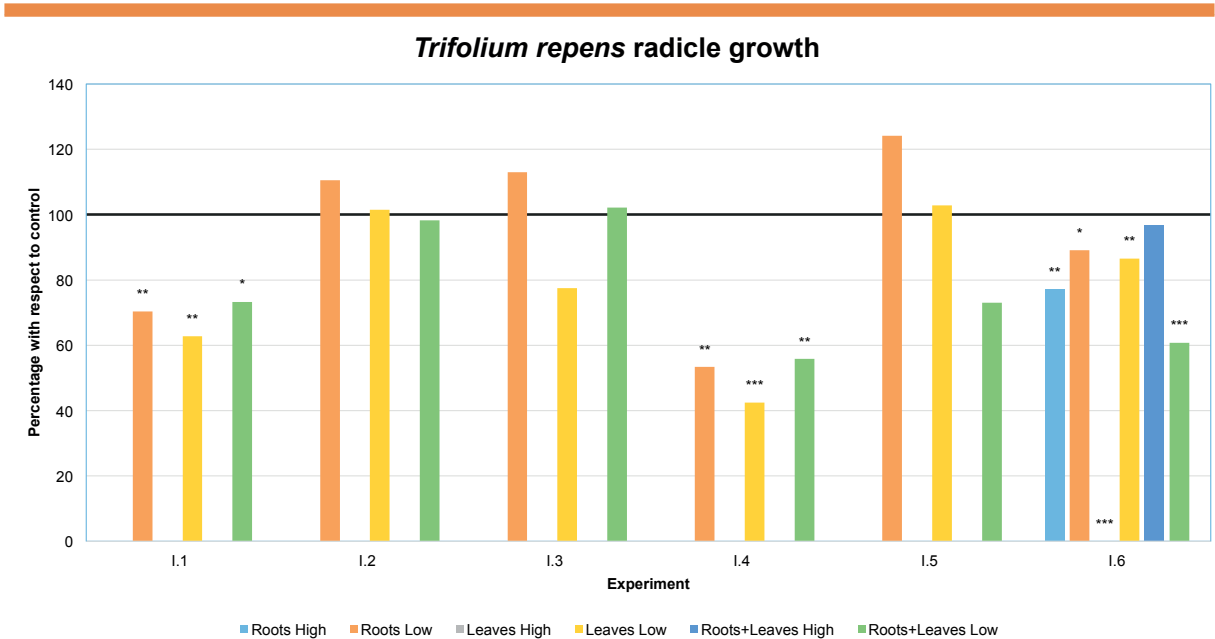


Figure 6.
Decomposition of Plant Residues I. Radicle growth bioassays. Species used: *Trifolium repens*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

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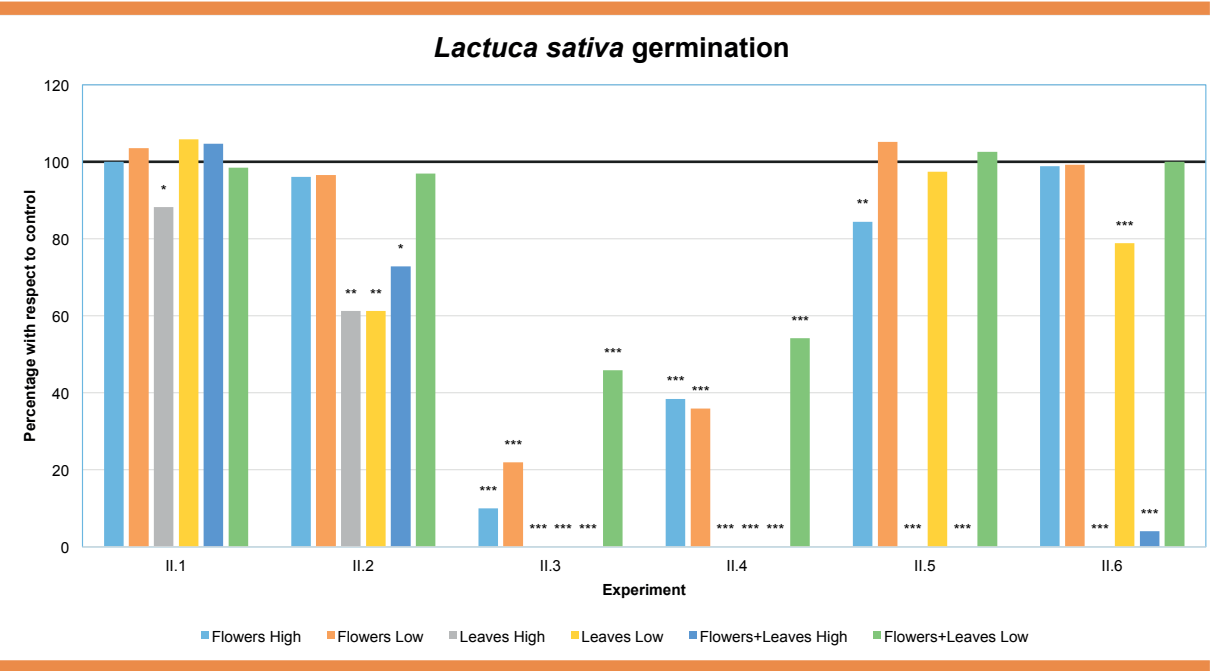


Figure 7.
Decomposition of Plant Residues II. Germination bioassays. Species used: *Lactuca sativa*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

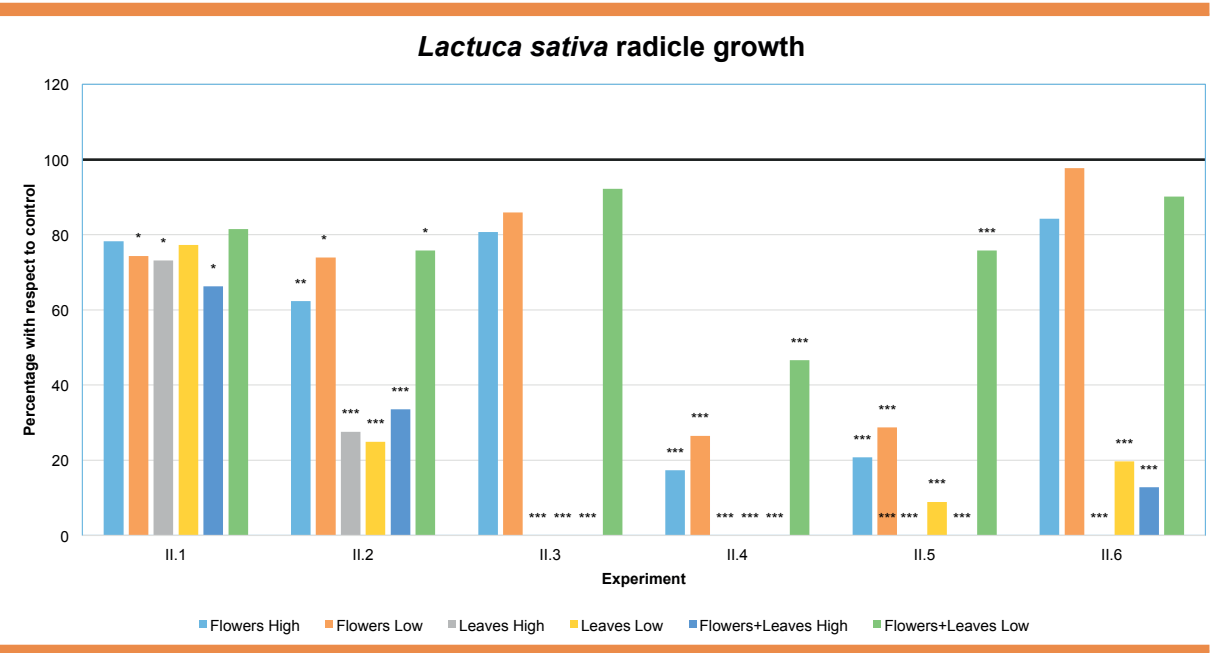


Figure 8.
Decomposition of Plant Residues II. Radicle growth bioassays. Species used: *Lactuca sativa*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

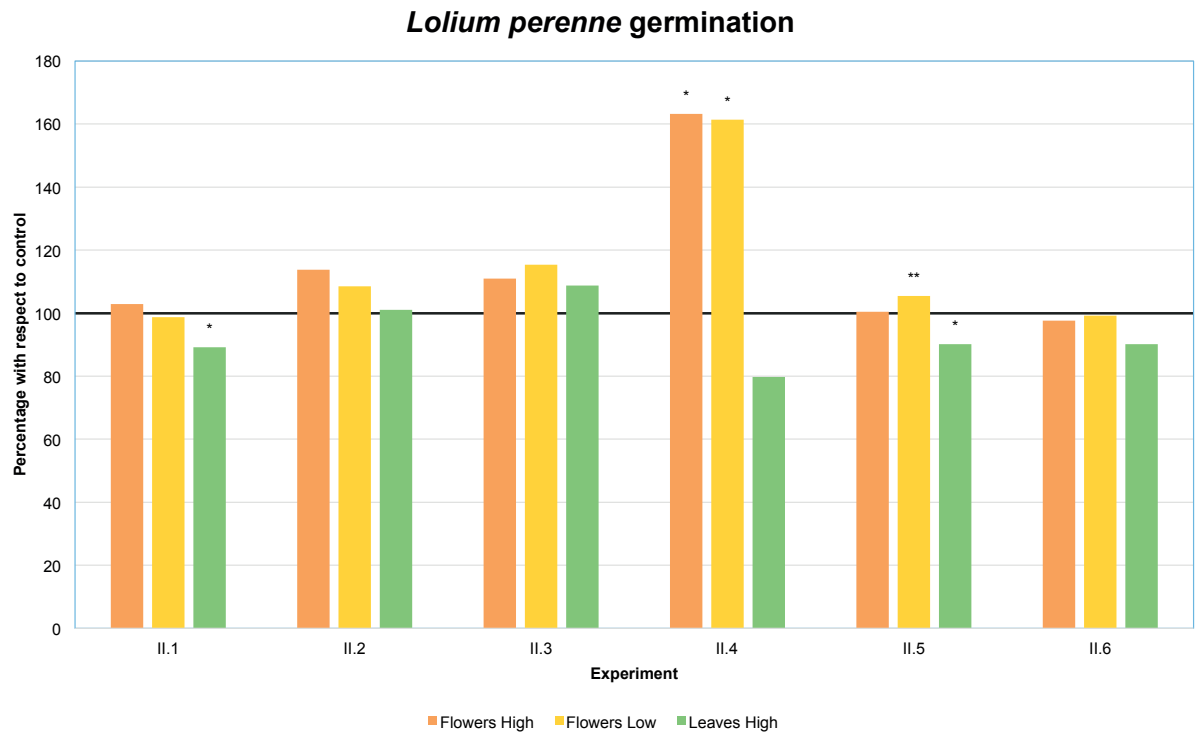


Figure 9.

Decomposition of Plant Residues II. Germination bioassays. Species used: *Lolium perenne*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

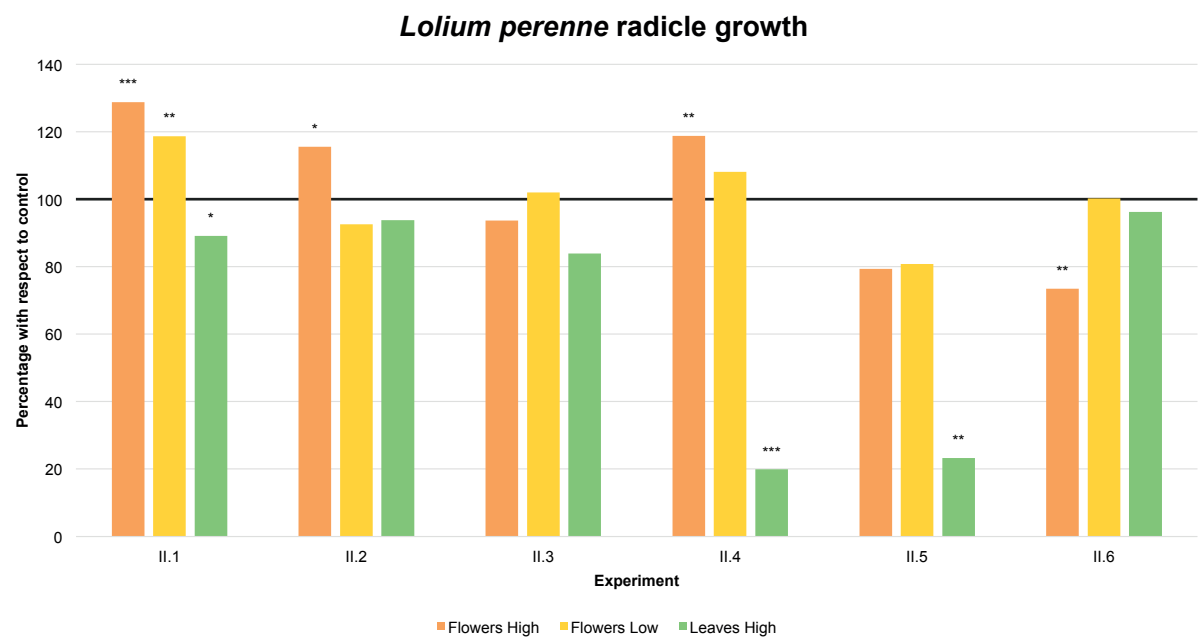


Figure 10.

Decomposition of Plant Residues II. Radicle growth bioassay. Species used: *Lolium perenne*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$

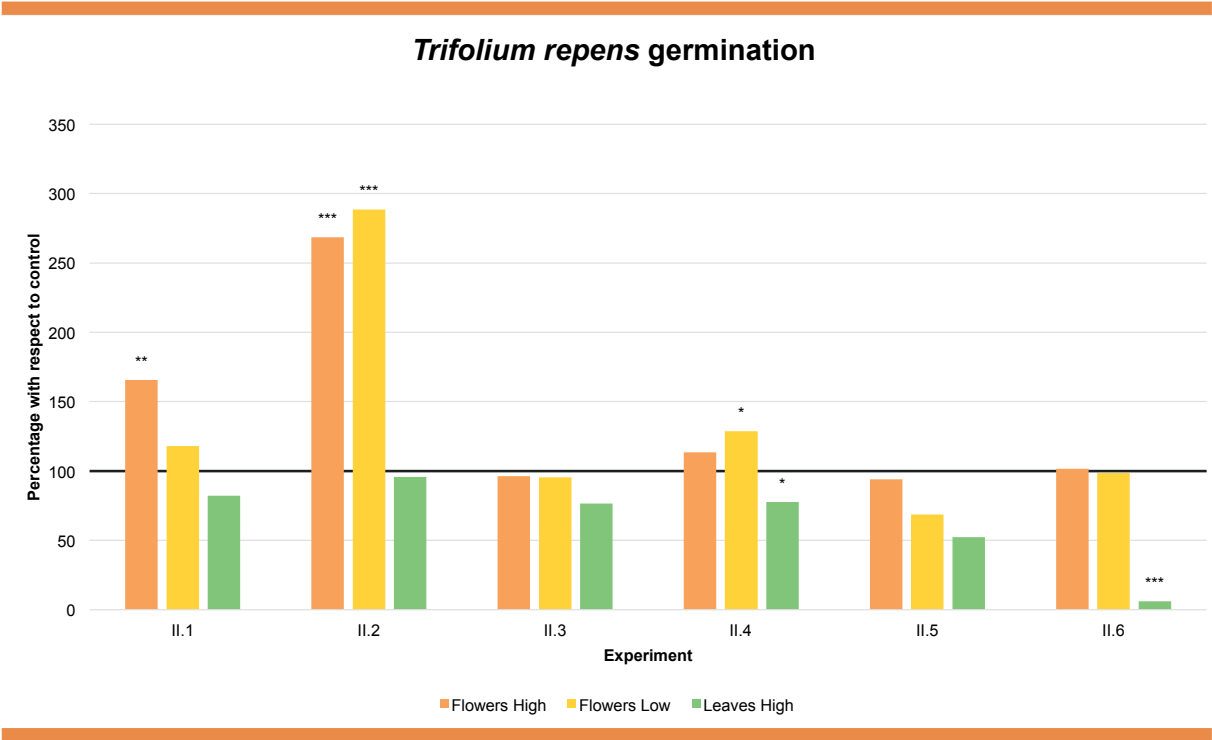


Figure 11.

Decomposition of Plant Residues II. Germination bioassays. Species used: *Trifolium repens*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

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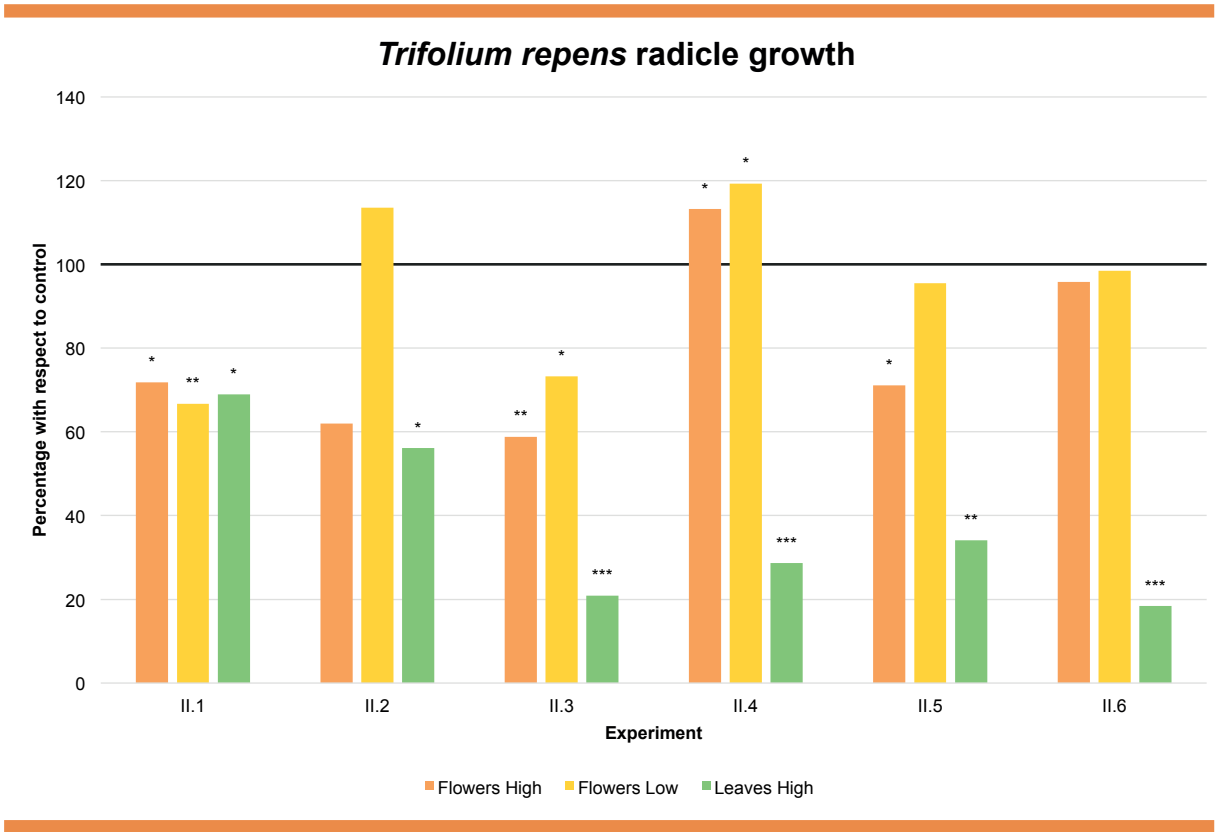


Figure 12. Decomposition of Plant Residues II. Radicle growth bioassay. Species used: *Trifolium repens*. All data are compared to the control. Statistical significant differences (ANOVA plus LSD) are shown: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$



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