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INDEX

- 9-17 Nascimento Franco, J.D., Rubido-Bará, M., Horjales-Luaces, M., Reigosa M.J.**
Allelopathy between seeds of the *Ulex* L. and *Genista* L. Genera
- 19-35 Carballeira, Alejo and Reigosa, Manuel J.**
Studies on the Allelopathic Potential of *Acacia dealbata* Link. Allelopathic Potential Produced During Litter Bag Decomposition of Plant residues in the Field





Allelopathy between seeds of the *Ulex* L. and *Genista* L. Genera

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ABSTRACT

Ulex europaeus, *Ulex gallii*, *Ulex micranthus*, *Ulex minor* and *Genista triacanthos* seeds collected at the Campus of As Lagoas, Marcosende, at the University of Vigo, were used in a series of experiments to check the competition between the five species, checking the effect of density. Results demonstrate that allelopathic effects are stronger between *Ulex* species, and the effects were density-dependent. The role of Allelopathy in the interactions between species was limited, but it could add to other competition effects (water, space, nutrients).

Keywords: Allelopathy, competition, density, *Ulex europaeus*, *Ulex gallii*, *Ulex micranthus*, *Ulex minor*, *Genista triacanthos*, germination, growth.

Introduction

According to Tukey (1969), the influence of a plant on the development of others has been a known phenomenon in agriculture for a long time; from the scientific point of view, the debate was started by DeCandolle in 1832, followed by the experiments carried out by Pickering (1917, 1919). In 1937, Molisch coined the classical terms that we still use nowadays, although the IAS has extended the definition of allelopathy to include more complex relations than the ones initially considered. However, we will stick here to the formulations of Molisch and Rice (1984).

Many of the interactions between plants are due to specific factors, such as the competition for water, light and nutrients, the susceptibility or immunity to plagues or illnesses, and the effects produced by other environmental stress types (Anthelm and Dangles, 2012; He *et al.*, 2013). We can define allelopathy as the interactions produced from one plant on another (including microorganisms)

through chemical compounds released into the environment; more often than not, the inhibitory effects have received more attention (Reigosa *et al.*, 1999; Tukey, 1969), but in certain conditions the effects can be stimulating (Rice, 1989). The most influent author from a historical point of view (Rice) defined allelopathy in 1989 as “any direct or indirect effect, harmful or beneficial, that a plant exerts on another by releasing secondary metabolites into the environment”.

The release of these chemical substances (known as allelochemicals or allelopathic agents) into the environment may occur in different ways; volatilization emanating from the aerial parts of the plant, lixiviation caused by rain water, fog and dew, root exudation and decomposition of plant residues (Anaya, 1999; Chou, 1986; Dallali *et al.*, 2014; Whittaker & Finney 1971). The decomposition of plant residues is the most important source of allelochemicals; this release process is not uniform, it changes depending on the ecosystem (Reigosa *et al.* 1999).

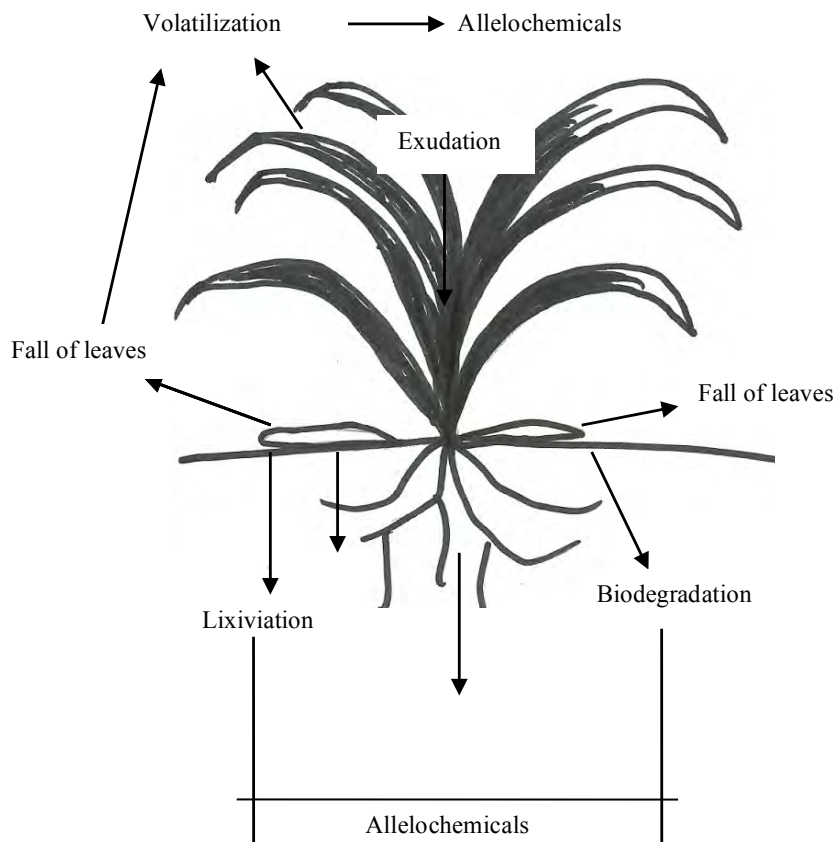


Figure 1.
Ways for the release of allelopathic compounds into the environment.

These compounds can interfere with the preservation, dormancy and germination of seeds, with the growth of seedlings and the vegetative development of adult plants. Furthermore, it can influence to a greater or lesser extent the competition between species, interfere with the natural regeneration or growth of new species in a given area that is conditioned by already existing plants and the compounds released into the environment. Seed germination tests have frequently been used as a bioassay for determining allelopathic activity, but very rarely has the interference of one seed on another during germination been analyzed (Chiapusio *et al.*, 1997; Wei *et al.*, 2017).

In many cases, the allelopathic effect does not lie on the capacity for germination – total percentage of germination in the final time (Labouriau, 1983), but on the germination speed or on other parameters of the process (Borghetti and Pessoa

1997; Haberman *et al.*, 2017; Hussain and Reigosa, 2014; Rodrigues *et al.*, 1999). The allelopathic effect can cause alterations in the distribution curve of germination, accelerating or decelerating the curve through the axis of time and changing its shape.

Some authors suggest that germination is less sensitive to allelochemicals than the growth of seedlings. However, the experimental quantification is much simpler, since for each seed of the phenomenon it is discrete, germinating or not germinating (Nielsen *et al.*, 2014). Allelopathic substances can induce the apparition of abnormal seedlings, this being one of the most common symptoms of radical necrosis (Pina *et al.*, 2009).

The aim of this study was to assess whether there is an allelopathic interaction between *Ulex europaeus* L, *Ulex gallii* Planch, *Ulex micranthus* Lange, *Ulex minor* Roth and *Genista triacanthos* Brot. seeds, during the process of germination.

Material and methods

During the experiment, *Ulex europaeus*, *Ulex gallii*, *Ulex micranthus*, *Ulex minor* and *Genista triacanthos* seeds collected at the Campus of As Lagoas, Marcosende, at the University of Vigo, were used.

The seeds were washed, scarified and distributed into small bowls, using 16 seeds of one species with 7 of another species in the following combinations: *Ulex minor* x *U. europaeus*; *Ulex minor* x *Ulex gallii*; *Ulex minor* x *U. miranthus*; *Ulex minor* x *Genista triacanthos*. In order to check if the effect of density is an influential factor, replicas were made with higher and lower amounts of each one of the two competing species. In total, 3 replicas were made for each combination, adding distilled water in a way that the water would enter into contact with all the seeds. Immediately the bowls were put in a germination chamber at a temperature of approximately 19.5 degrees and for a photoperiod of 14 hours of light and 10 of darkness. The number of germinated seeds was counted from the 4th day, considering a seed is germinated when there is a protrusion of the reticule of at least 1mm. The experiment lasted 21 days.

Statistical analysis

The data were standardized in percentage according to the control, taking into account the different number of seeds present in each treatment. The averages and standard deviations of the three replicates used in the different tests were calculated. To check the differences induced by allelopathic interaction, the different treatments were compared with the control by applying a one-way variance analysis, using the least significant difference test (LSD) with a significance level of 0.05. To test the effect of seed density, a variance analysis was performed using a Student's t-test for independent samples with a confidence level of 95%, assuming equality of variances.

The equality of variances was evaluated with a Levene test. In the case of non-homogeneous variances, the non-parametric Kruskal-Wallis test (Sokal e Rohlf, 1995) was applied. The statistical analysis was performed using the SPSS 14.0 program for Windows.

Implemented treatments:

	Number of seeds	
Treatment 0 (control)	15	
	<i>Ulex minor</i>	<i>Ulex europaeus</i>
Treatment 1-16*7	16	7
Treatment 1-7*16	7	16
	<i>Ulex minor</i>	<i>Ulex gallii</i>
Treatment 2-16*7	16	7
Treatment 2-7*16	7	16
	<i>Ulex minor</i>	<i>Ulex micranthus</i>
Treatment 3-16*7	16	7
Treatment 3-7*16	7	16
	<i>Ulex minor</i>	<i>Genista triacanthos</i>
Treatment 4-16*7	16	7
Treatment 4-7*16	7	16

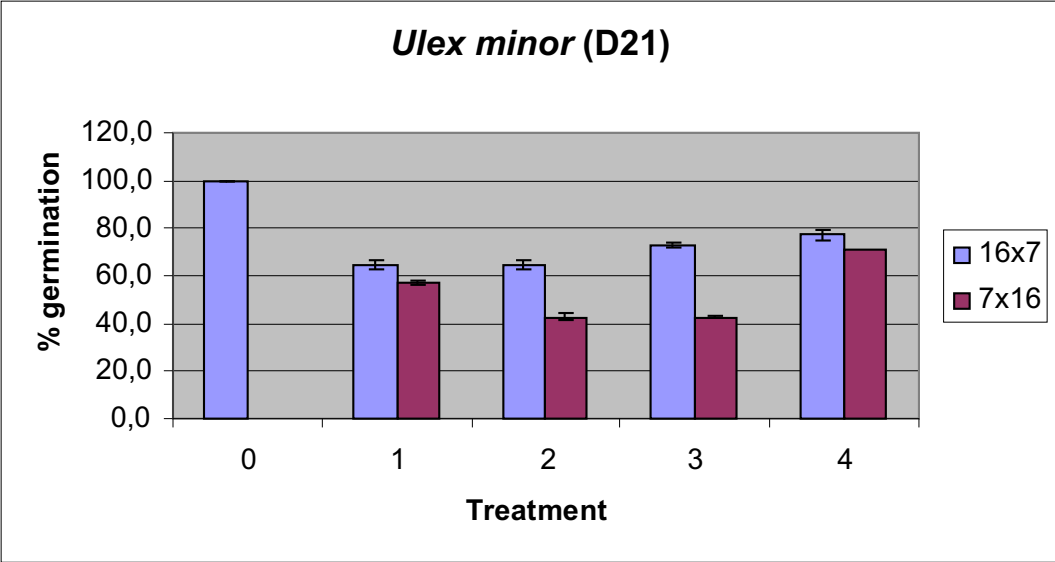


Figure 2. Germination percentages of *Ulex minor* on dish in isolation (treatment 0) and in the tests with other species (1- *Ulex europaeus*. 2- *U. gallii* 3- *U. micranthus* and 4-*Genista triacanthos* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate the significant differences at the 5% level with respect to the control.

Results and discussion

Figure 2 Germination percentages of *Ulex minor* on dish in isolation (treatment 0) and in the tests with other species (1- *Ulex europaeus*. 2- *U. gallii* 3- *U. micranthus* and 4-*Genista triacanthos* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate the significant differences at the 5% level with respect to the control.

Treatments 1 and 2, for the two densities of *Ulex minor* exhibit significant differences at the level of 95% with regard to the control. Therefore, *Ulex minor* decreases its ability to germinate in 35.4% in competition with moderate densities of *Ulex europaeus* and *Ulex gallii* seeds and further reduces germination when there is a high density of seeds of the same species, 42.87% against *Ulex europaeus* and 57.13% against *Ulex gallii*. When the *Ulex minor* seeds are in competition with *Ulex micranthus*, the germination of the first ($p \leq 0.05$) is reduced only when the density of *Ulex micranthus* is high. In this case, *Ulex minor* does not reach 50% germination. Although *Ulex minor* shows a decrease of almost 30% in germination when in competition with *Genista*

triacanthos seeds, no significant differences with regard to the control are observed.

In this experiment, the test performed with *Ulex micranthus* (treatment 3) is remarkable because it establishes significant differences to the level of 95% between the two tested densities. That is to say, the inhibition of the germination of *Ulex minor* is dependent on the density of *Ulex micranthus* present in the test.

Figure 3 shows the germination results of *Ulex europaeus* compared with two densities (high and moderate) of *Ulex minor* at the end of the treatment (21 days after the start of the experiment). The two densities diminish the germination of *Ulex europaeus*: 46.43% before the high density of *Ulex minor* and 38.23 % when the density of this last one is lower.

The corresponding results regarding the effect *Ulex minor* exerts over the germination of *Ulex gallii* are shown in **figure 4**.

Both the high density and the low density of *Ulex minor* in the test cause the inhibition of the

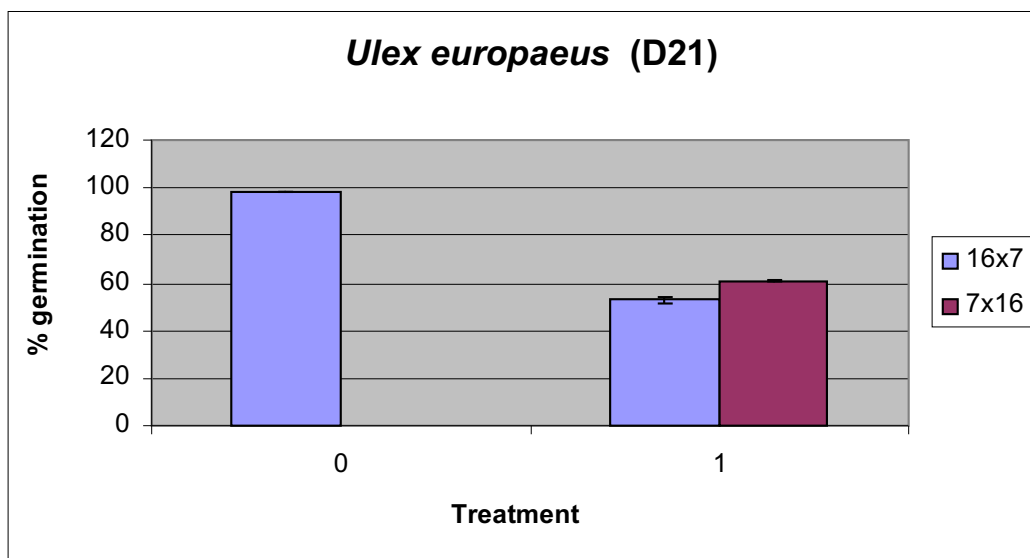


Figure 3.

Percentages of germination of *Ulex europaeus* on dish after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

germination of *Ulex gallii* ($p \leq 0.05$), reducing it to 52.90% when the density of *Ulex minor* is higher and in 40.50% when the density is lower. Despite the differences in the measures obtained, no significant differences were found between the two used densities (but there is with regard to the control).

Figure 5 shows the results obtained for the *Ulex micranthus* species. The presence of *Ulex minor* in the test produces strong inhibitions in the germination of *Ulex micranthus* ($p \leq 0.05$), producing a stronger effect in the highest density of *Ulex minor* that causes an inhibition superior to 50%.

Figure 5 Percentages of germination of *Ulex micranthus* on plate after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

The effect of the *Ulex minor* seeds on the germination of *Genista triacanthos* is shown in **figure 6**. Despite the inhibitions that appear at

almost 50% in the two tested densities, no interaction ($p \leq 0.05$) is observed between the seeds of both species.

Figure 6 Percentages of germination of *Genista triacanthos* on dish after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

Theoretically, all plants are potentially able to synthesize compounds with allelopathic properties. This ability is less prevalent in cultivated plants and their commercial varieties. Meanwhile, this characteristic was more common in the wild precursors than in the current cultivated plants, which suffered artificial selection to obtain the best agronomic conditions, but overlooking their ability to chemically defend against competitors or against insects and pathogens (Bansal & Bhan, 1993).

The available information shows that all parts of plants may contain allelopathic substances and that these chemical substances are not distributed evenly

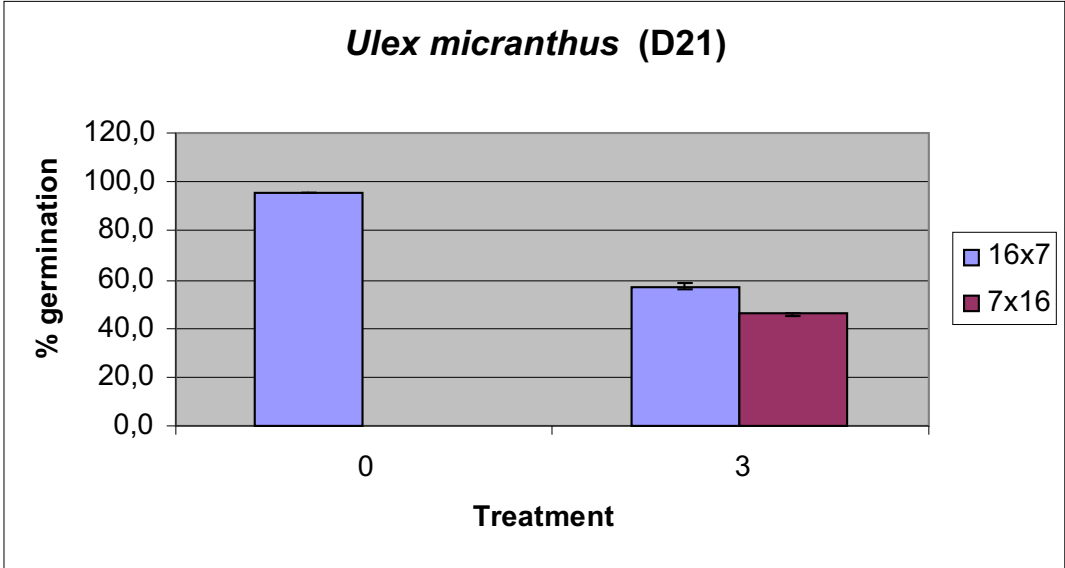


Figure 4. Percentages of germination of *Ulex gallii* on dish after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

across the different organs of the plants. There could be a higher concentration in the leaves or in the roots, or in the bark of the trunk of tree species o even in the seeds (Souza Filho *et al.*, 1997; Corcera *et al.*, 1992; Heisey, 1990; Rao, 1990; Friedman & Waller, 1983). In different bioassays, these substances were found in leaves, flowers, rhizomes, roots, fruits, seeds and barks of different plant species (Smith & Martin, 1994; Friedman & Waller, 1983; Cope, 1982; Young & Bartolomew, 1981; Putnam & DeFrank, 1982; Heisey, 1990; Souza Filho, 1995). In this project, seeds were the source of potentially allelopathic water soluble substances.

Seeds are an important means of production of new individual plants in the countryside. The study of the seed bank is usually quite complicated, considering the enormous capacity many plants have to produce seeds (up to 20,000 per plant and year). The seeds accumulate on the ground with a viability of up to 70 years and the expulsion of the seeds can be up to 5 m away from the plant (Cubas, 1999; Ivens, 1983), with quite varied posterior dispersion methods and capable of long distance dispersion. Considering that in this study

the allelopathic effects vary depending on the number of seeds, the knowledge on the species that form the herbaceous layer and the potential seed production is seen as an important tool to be considered in studies in the field attempting to understand the biological activity of chemical substances with allelopathic activity.

In superior plants, compounds with highly chemically diversified allelopathic properties have been found and their quantity and composition usually varies on the species been studied, the phenological state, the part of the plant and the different environmental situations (Putman, 1985). Various chemical substances related to allelopathic ability have been described in detail by authors such as Rice (1984) and Putnam & Tand (1986). Whittaker & Feeny (1971) classified these secondary products in five main categories: terpenoids, steroids, alkaloids, acetogenins and phenylpropanoids. Rice (1984) presented a broader classification of these compounds including quinones, tannins, flavonoids and others. In rainy climate plants the main metabolites with an allelopathic ability are usually water soluble, with great preponderance of phenolic substances.

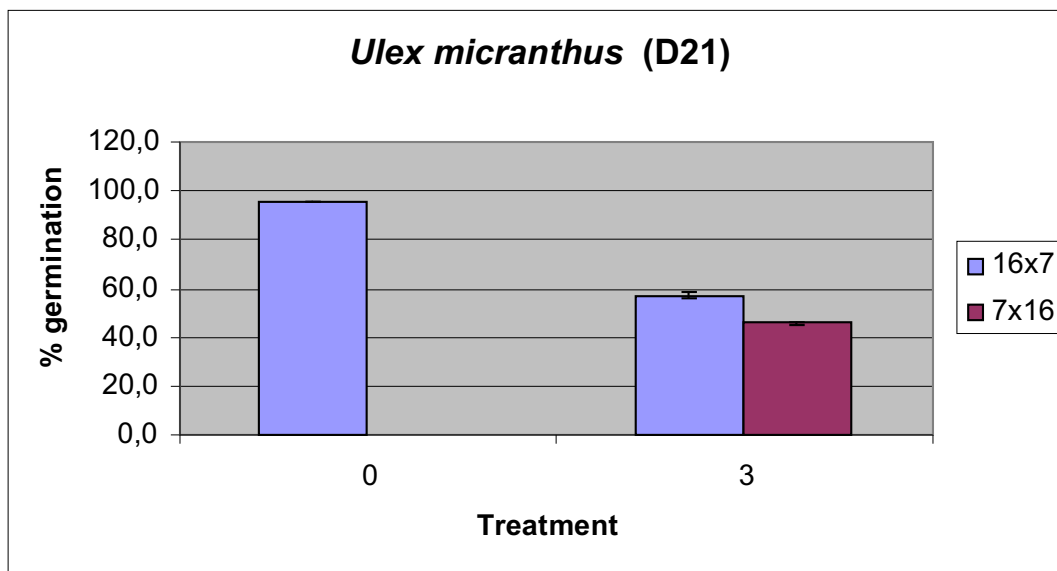


Figure 5.

Percentages of germination of *Ulex micranthus* on plate after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

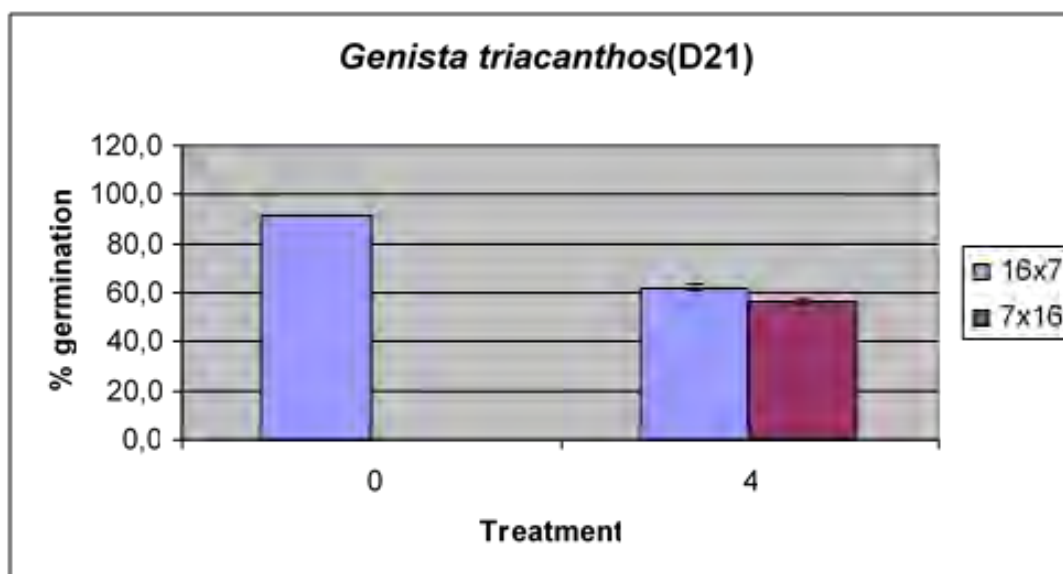


Figure 6.

Percentages of germination of *Genista triacanthos* on dish after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

Conclusion

Allelopathy is another process in the competition between species. This work is a contribution to the study of the competition between three species of the genus *Ulex* and *Genista triacanthos* that are natural to Galicia and live together by means of allelopathic effects between seeds in the germination process. The high phenotypic plasticity (Delerue *et al.*, 2013) should be taken into account.

From the results obtained in this experience we can conclude that the allelopathic pressure during the germination process of these species is superior between species of the same genus and in some cases it depends on the density of the seeds in the tests (germination of *Ulex minor* in the presence of *Ulex micranthus*).

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Studies on the Allelopathic Potential of *Acacia dealbata* Link. Allelopathic Potential Produced During Litter Bag Decomposition of Plant residues in the Field

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ABSTRACT

Temporal evolution of the phytotoxic capacity of lixiviates obtained from residues of *Acacia dealbata* in decomposition was studied. Such residues were decomposed in the soil in the field by means of the litter bags technique. The phytotoxic capacity was studied by means of bioassays of germination and growth of *Lactuca sativa*, *Dactylis glomerata*, *Lolium perenne*, *Trifolium repens* and *Trifolium pratense*.

Two series of experiments were carried out, by working with naturally released plant residues with two different compositions. The results demonstrate that the high allelopathic capacity of decomposing residues from *A. dealbata*, although significant toxicity in bioassays is a bit different to the field conditions. The phytotoxic capacity when flowers are present is higher than bioassaying other plant materials.

Keywords: Allelopathy, Litter Bags, *Acacia dealbata*, *Lactuca sativa*, *Lolium perenne*, *Trifolium repens*, *Trifolium pratense*, Plant Residues Decomposition.

Introduction

There are different ways of allelochemical substances release to the environment (Aslam *et al.*, 2017; Ballester, 1971; Bauer *et al.*, 2012; Kaur *et al.*, 2012; Reigosa *et al.*, 1999; Rice, 1984; Tukey, 1966), but it has been demonstrated that the decomposition of residues is in general the greatest source of allelopathic substances (Araniti *et al.*, 2016; Ballester, 1972; Del Moral and Cates, 1971; Goel *et al.*, 1994).

Plants residues have compounds that on being released directly or through the microbial decomposition under determined edaphic conditions can have allelopathic capacity (Kaminsky, 1981; Wang *et al.*, 2015; Whitehead, 1964). Likewise allelopathic substances can appear in the soil coming from aerial parts leaching or radicle exudation (Chaves *et al.*, 2016; Patrick, 1971; Rovira, 1969; Wei *et al.*, 2017) or even from the swept down dew of volatile compounds released to the environment (Aguilera *et*

al., 2015; Del Moral and Muller, 1969; Topuzovic *et al.*, 2015). Therefore the most correct way to know the real toxicity that might appear during the vegetal residues decomposition shall be by allowing that vegetal residues will become decomposed in the same soil where we suspect that there are allelopathic nature phenomena (Chou, 2010; Molina *et al.*, 1984).

Works about the accumulation, persistence and stability of allelopathic substances in the soil are still scarce (Li *et al.*, 2011; Rice, 1984), but it has been proved the importance of different environmental factors (Blum *et al.*, 1993; Dawkins and Esiobu, 2016; Guenzi and McCalla, 1966; Inderjit and Dakshini, 1994; Juste *et al.*, 1985; Muller and Chou, 1972; Patrick and Koch, 1958; Reigosa *et al.*, 1984; Stevenson, 1967). From among the most important factors are the microorganisms (especially fungi and bacteria) of the soil (Blum *et al.*, 1994; Dawkins and Esiobu, 2016; Grant, 1976; Jalal and Read, 1983; Kung, 1971; Leao, 1976; Tack *et al.*, 1972; Turner and Rice, 1975).

Works about the temporal evolution of phytotoxicity in soils are also scarce (Carballeira and Cuervo, 1980; Casal *et al.*, 1985; Kaur *et al.*, 2012; Lodhi, 1975, 1976; Nilsson *et al.*, 1998; Rice, 1984; Robinson, 1971). Through some works it can be verified that toxins suffer from a fast diachronic evolution, both in quality and quantity, which have very fast activation and deactivation rates (Jalal and Read, 1983; Reigosa and Carballeira, 1999; Reigosa *et al.*, 1984). Important toxins accumulation have been found during a short but physiologically decisive period taking into account the vital cycles both from the emitting and the receiving ones, so correlations between the vital cycle stage of compounds release and the climatic conditions, particularly the ones related to washing by rain are found (Dastagir and Hussain, 2015; Leon, 1976; Mitchell, 1968; Quarterman, 1973; Squires and Trollope, 1978; Tukey, 1969; Turner and Quarterman, 1975).

This work studies the allelopathic potential capacity of residues coming from the *Acacia dealbata* as they decompose in the soil of the mimosa area by means of the litter bags technique and subsequent washing with water of the decomposition residues. It has also followed the evolution, all a year round, of the fallen leaves into the piece of ground. The role of allelopathy in the invasive potential of *Acacia dealbata* has been suggested previously (Lorenzo *et al.*, 2008, 2010).

Material and methods

In order to have an idea about the quantity and composition of litter produced by *A. dealbata* fallen down on the piece of ground studied during a year, it was carried out a collection at the same time than the collection of natural washes described in Carballeira and Reigosa (1999). To do it, eight fallen leaves collecting devices were placed into the piece of ground at random, in the area where the sole present arboreal species was the *A. dealbata*. Such collecting devices were horizontal aluminum rings of 1 mm diameter lifted up 1 m. by means of a pole of the same metal which was sunk into the soil. From this ring a sort of conicalshaped funnel was hanging that is made up of mesh cloth having an opening of 0.1 mm. Such funnel was enlarged (85 cm) to avoid fallen leaves loss due to the wind or bounces and was fastened to the soil by means of a wire. These collecting devices were emptied out 26 times during one year. Once collected, the material it was carried to the laboratory where its dry weight was determined. Likewise a previous study about the flower fall (Reigosa *et al.*, 1984) is available.

Two decomposition bioassays of vegetal residues in the soil were carried out: one without flowers, the second including flowers decomposing.

In order to get the vegetal material five plastic square were placed randomly in the mimosa area as described in Reigosa and Carballeira (1999), by collecting the whole of fallen leaves after 15 days. So the collected residues have suffered from natural abscission, with which we meet the aim of approaching the natural conditions. It is necessary to point out that the material that was divided by hand offers a different composition as regards the one falling in a natural way (Attiwill *et al.*, 1978). Such differences will affect in a quantitative and qualitative manner both the released products during decomposition and the own decomposition rate.

In the laboratory it was determined the dry weight of three aliquots that allowed us to calculate the fresh weight equivalent to 30 g of dry weight, this being the one that after homogenizing all of the collected material, is closed into each one of 35 mesh bags. The bags were built of nylon fabric, the size of each one is 20x10 cm and the mesh is 2 mm.

Bocock (1964) with *Fraxinus excelsior*, MacCauley (1975) with *Eucalyptus pauciflora* and Gloaguen and Touffet (1980) with *Fagus sylvatica* found no significant difference concerning the decomposition rates studied with bags whose mesh diameters fluctuated between 0.5 and 2 mm and between 2.5 and 5.

The bags prepared as above were placed in five groups of seven distributed at random, by sinking them in the upper layer of the fallen leaves covering the soil. The litter bags with the materials (first without flowers, later with flowers) were buried and samples were recollected on next day, as well as 7, 15, 30, 45, 60 and 90 days later, and corresponding bioassays (Litter Bags I and Litter Bags II) were carried out. On each fixed day a bag belonging to each of the five points was taken at random by separating the material in two portions: one of them for weight determination by means of the dry weight percentage calculation and the other one to study its phytotoxicity. With this second portion, the extracts used in bioassays were prepared. The vegetal material under decomposition was saturated with distilled water, was let to macerate during 24 hours in the dark at 20° C and was washed with distilled water by means of slow spraying up to get 100 ml of extract for each 100 g of dry weight. The aqueous extract so obtained is to be named 1/1 from now on, and after that three other solutions were prepared by adding distilled water in sufficient quantity to get a concentration 1/2, another 1/10 and a third one 1/50 that are so named.

From each extract pH and conductivity are measured and bioassays are carried out with the *Lactuca sativa* (var. Great Lakes), *Lolium perenne*, *Dactylis glomerata*,

Trifolium repens and *Trifolium pratense* by sowing five plates with 50 seeds each, there including the control made with distilled water. On each Petri plate two Whatmann 3 MM paper sheets are placed and on them the seeds, by watering them with 7.5 ml of the corresponding solution. The plates are located on stove at 28° C and 80% humidity during 48 hours in the case of *L. sativa*, 96 h. for *T. pratense* and *T. repens* and 120 h for *D. glomerata* and *L. perenne*. After this time, the plates were placed into a refrigerator at least for 4 h. with the aim of stopping their growing and the germinated seeds were counted and the radicles length was measured.

Statistical Analysis. The results for each experiment were submitted to analysis of variance and LSD multiple range test in order to know the statistical meaning of differences among treatments (Sokhal and Rohlf, 1979) after demonstration of normal distribution (Kolmogorov-Smirnov test) and homoscedasticity (Levene test).

Results and discussion

The values of humidity and dry weight percentage for each assay are shown in the Tables 1 and 2 while the pH and conductivity pH are shown in the Tables 3 and 4. The values of DW/FW have been correlated with the

meteorological data and there is, as expected, a good correlation between the rainy periods and the hydration of the plant decomposing materials (data not shown). The obtained values of pH and conductivity do not allow understanding the inhibitions as being explained by osmotic potential nor pH. All pH and conductivity values would permit normal germination and radicle growth, so the statistically significant inhibitions and stimulations must be due to the activity of the dissolved allelochemicals (Halvorson *et al.*, 2017). Generally, the extracts obtained in the second series of experiments (Litter Bags II) have a higher electrical conductivity, so more concentration of solutes is expected.

Litter bags I.

The results corresponding to germination and radicles growth of each one of the assayed species are shown in the figures 1 to 5 (germination) and 6 to 10 (radicle growth).

Figure 1 shows the effects along time of the four assayed concentrations on germination of *Lactuca sativa*. Although this species is quite sensitive to many allelochemicals, the effects have been very limited, showing some inhibition of germination at the beginning of the experiment (1 and 7 days after burial) and after 30 days. No significant effect appears later than 30 days after burial.

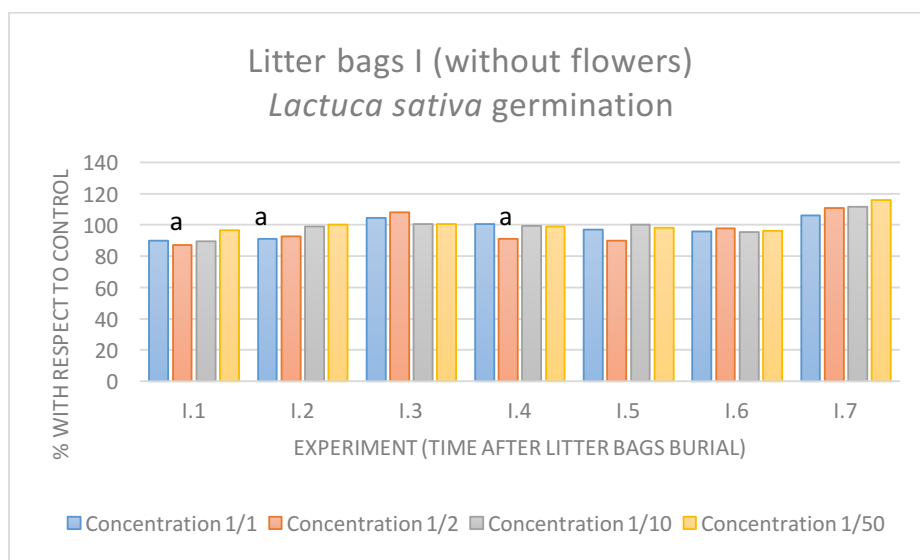


Figure 1.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on germination of *Lactuca sativa*. a, b, c, significant $p < 0.001$, significant $p < 0.01$, significant $p < 0.05$ respectively (LSD test).

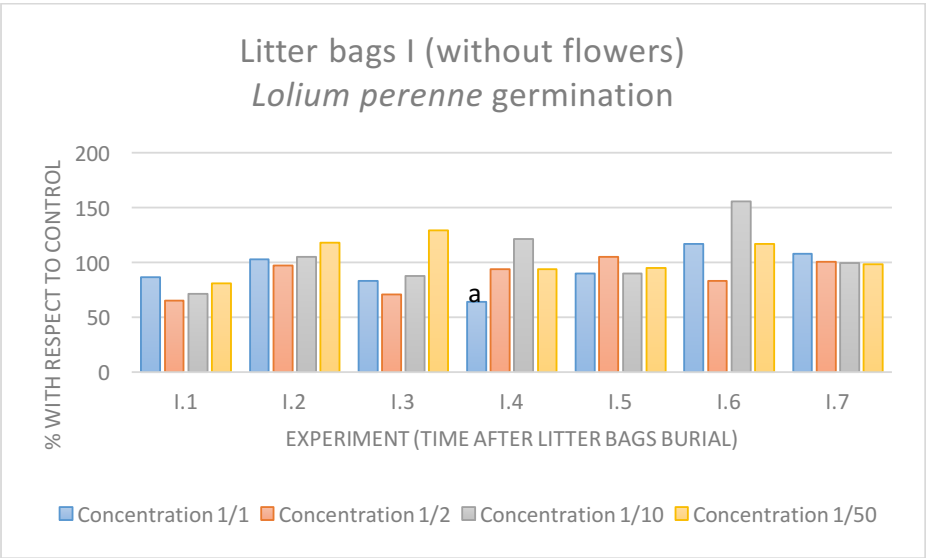


Figure 2.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment I, litter not containing flowers. Effects on germination of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Figure 2 includes the data of germination of *Lolium perenne*. This was the less affected species, showing only a slight inhibition 30 days after burial. No significant effect appeared at the beginning of the experiment (recently fallen leaves and plant materials) nor at the end of the experiments, when litter are more decomposed.

Figure 3 shows the effects of the litter leachates on germination of *Dactylis glomerata*. Apart from a not very

important effect of the concentration half concentration depressing germination in time I.2 (7 days after the burial) the only consistent effect is in the last experiment (I.7, corresponding to 90 days after burial) with a general depression of germination even in the lowest concentrations. *D. glomerata* germination was not affected in the initial phase of decomposition in the field, and only that final effects are noteworthy, just reflecting a more intense germination inhibition when litter is more decomposed.

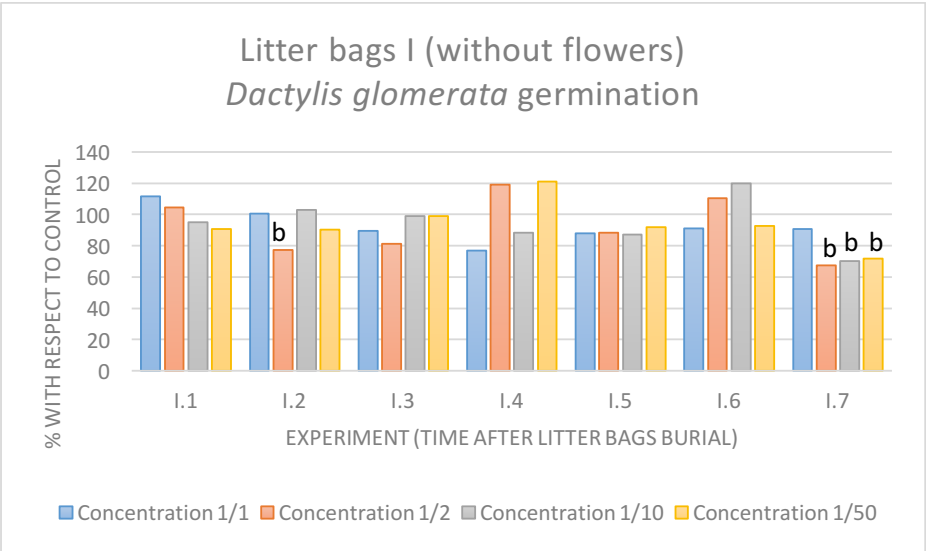


Figure 3.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment I, litter not containing flowers. Effects on germination of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

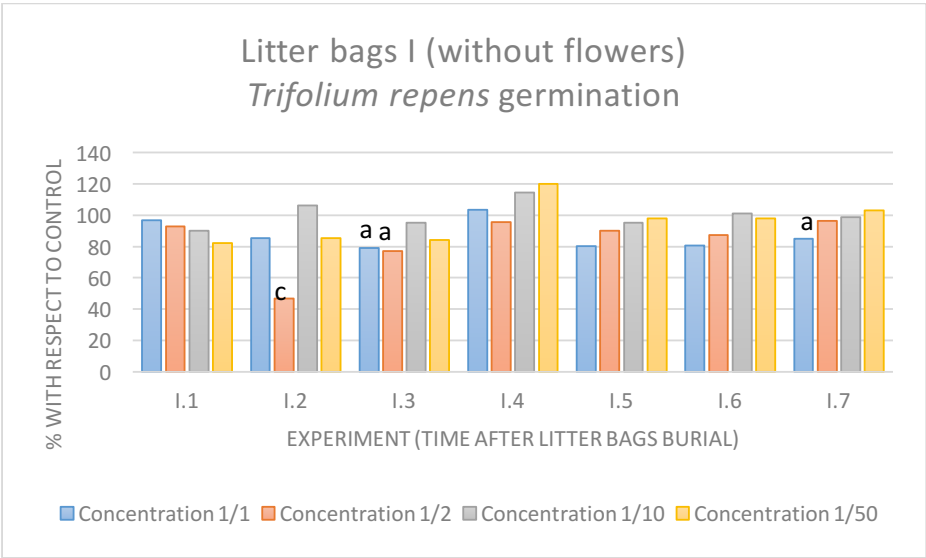


Figure 4.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on germination of *Trifolium repens*. a, b, c, significant $p < 0.001$, significant $p < 0.01$, significant $p < 0.05$ respectively (LSD test).

Figure 4 shows the effects of the leachates on *Trifolium repens* germination. Again, the germination is not very intensively affected, but in this case, the inhibitory effects are only significant in the experiments I.2 and I.3 corresponding to 7 and 15 days after burial. A small significant inhibition is also noted when testing the highest

concentration in the last experiment, but less intense than in other species.

Figure 5 includes the effects produced on germination of *Trifolium pratense*. This species is not inhibited, albeit some minor effects at the beginning of the decomposition process.

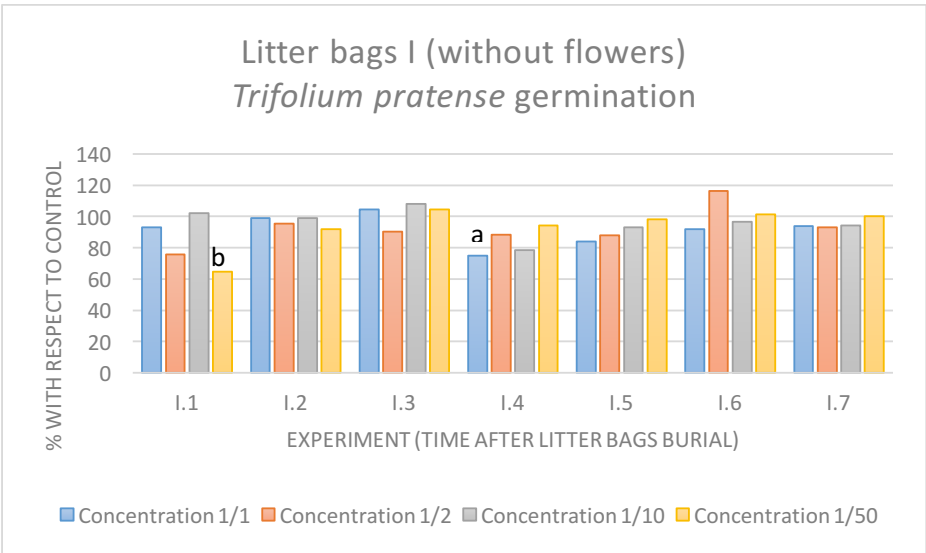


Figure 5.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on germination of *Trifolium pratense*. a, b, c, significant $p < 0.001$, significant $p < 0.01$, significant $p < 0.05$ respectively (LSD test).

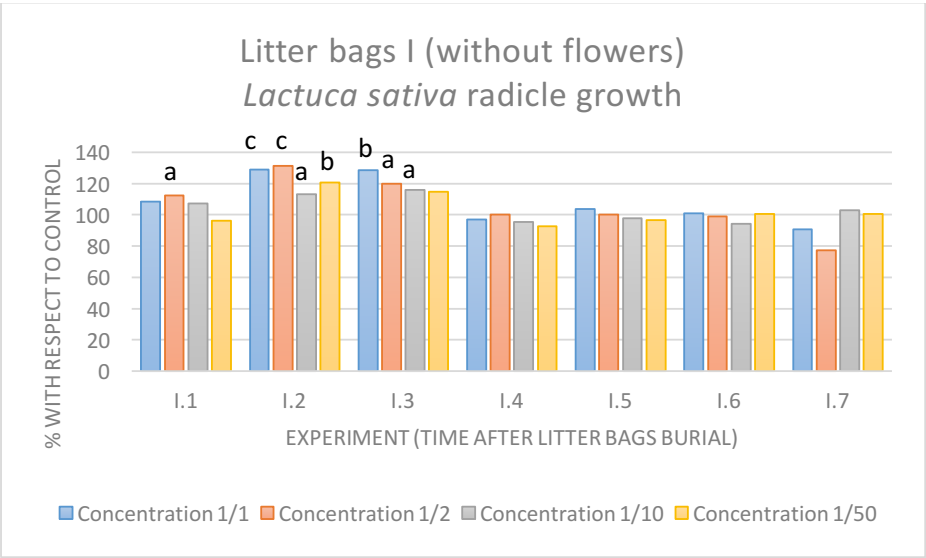


Figure 6. Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Lactuca sativa*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Figure 6 shows the effects of the leachates of *A. dealbata* plant litter decomposing in the field on radicle growth of *Lactuca sativa*. Again, the effects are not very marked, just showing slight promotion of radicle growth at the beginning (1, 7, and 15 days after burial). Combining with the results of germination (fig. 1) we can affirm that only in the first stages of the

decomposition process there is some inhibition, and that the effects are quite limited.

Figure 7 shows the effects on *Lolium perenne*. In this species, there are strong inhibitions in the first two dates (1 and 7 days after burial) and some inhibition in the highest concentration at the end of the experiment (90 days after burial).

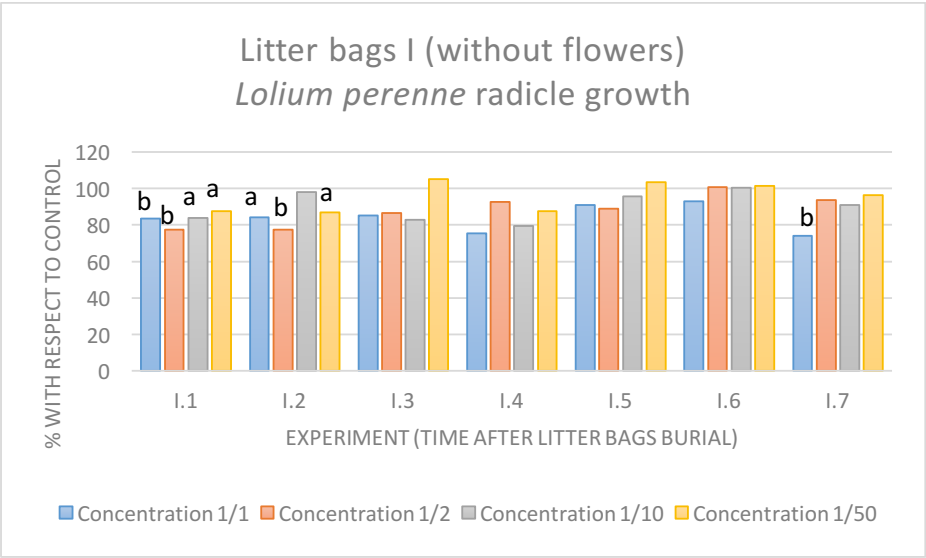


Figure 7. Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

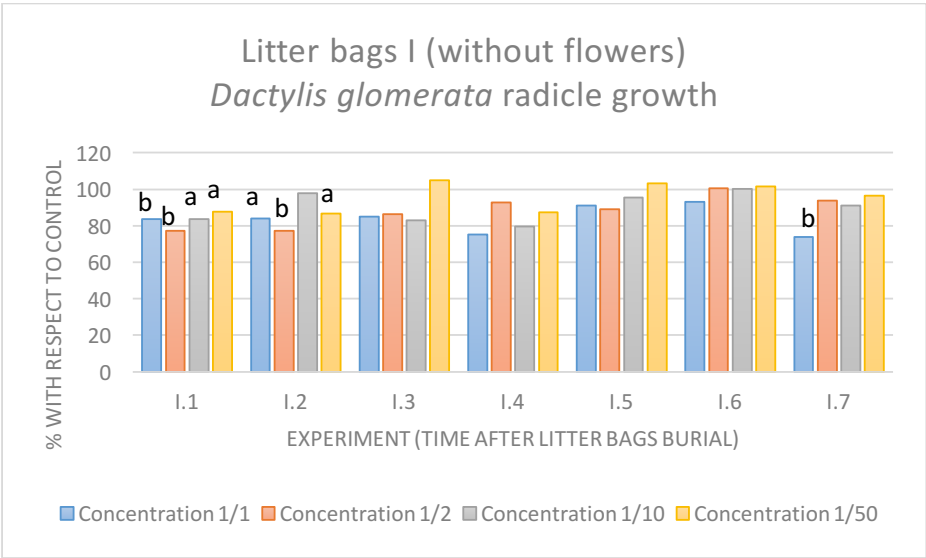


Figure 8.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Figure 8 pictures the effects of the *A. dealbata* decomposing litter leachates on *Dactylis glomerata* radicle growth. Results are similar to those obtained with *Lolium perenne*, with strong significant inhibitions at the beginning (1 and 7 days after burial) and at the end (90 days after burial, in this case only the strongest concentration shows statistical significant effects).

Figure 9 includes the data about *Trifolium repens*, which has been strongly inhibited at all times except in experiment I.6 corresponding to 60 days after burial, when no effect is demonstrated. This means that *T. repens* is more sensitive to the released allelochemicals, and that this species is reacting both to the beginning of the experiment and to the final process of decomposition.

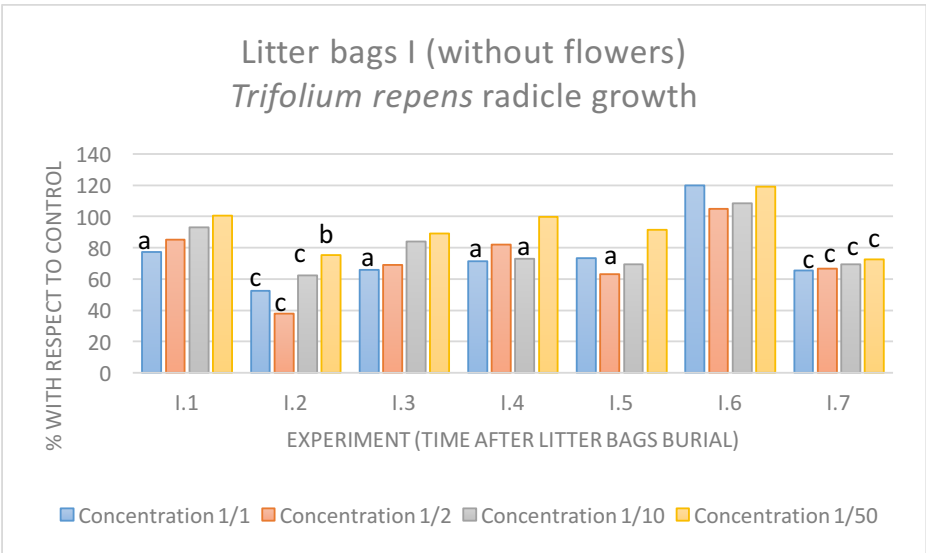


Figure 9.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Trifolium repens*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

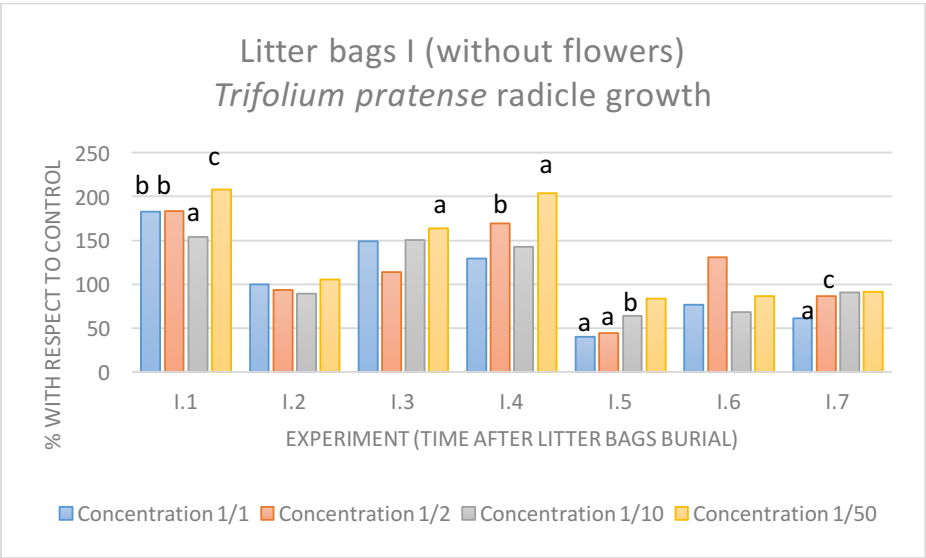


Figure 10.
 Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Trifolium pratense*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Figure 10 shows the effects on *Trifolium pratense* radicle growth. In this case, there is a significant increase in radicle growth at the beginning of the experiment (1 day after burial) with stronger inhibitions at the middle and the end of the experiments (15, 30, 45, 90 days), with the strongest inhibition at the end.

Germination is not very affected when the litter do not contain flowers, although we can affirm that the effects of

the leachates of litter being decomposed in the soils could be added to the effects of the decomposed materials and the leachates coming from the living tress, as previously demonstrated (Reigosa and Carballeira, 2017a)

Radicle growth is only inhibited slightly in the first series of experiments, too. In addition, both data (germination and radicle growth show a picture of weak although significant inhibitory effects.

Table 1. Humidity and dry weight percentage in the assays corresponding to Litter Bags I. Prior to the burial of the plant materials, the ratio dry weight/fresh weight was 65.58%

Experiment	Fresh weight	Dry weight	%
I.1	16.6	6.2	37.70
I.2	16.3	5.7	34.72
I.3	17.7	5.4	30.51
I.4	16.1	6.6	41.12
I.5	7.8	3.7	47.69
I.6	10.2	6.0	58.43
I.7	14.4	5.6	38.72

Table 2. Humidity and dry weight percentage in the assays corresponding to Litter Bags II. Prior to the burial of the plant materials, the ratio dry weight/fresh weight was 54.24%

Experiment	Fresh weight	Dry weight	%
II.1	13.2	7.5	56.67
II.2	16.2	6.7	40.24
II.3	14.6	6.2	42.47
II.4	30.1	8.3	27.64
II.5	30.0	8.6	28.6
II.6	27.4	8.1	29.49
II.7	11.9	4.6	38.66

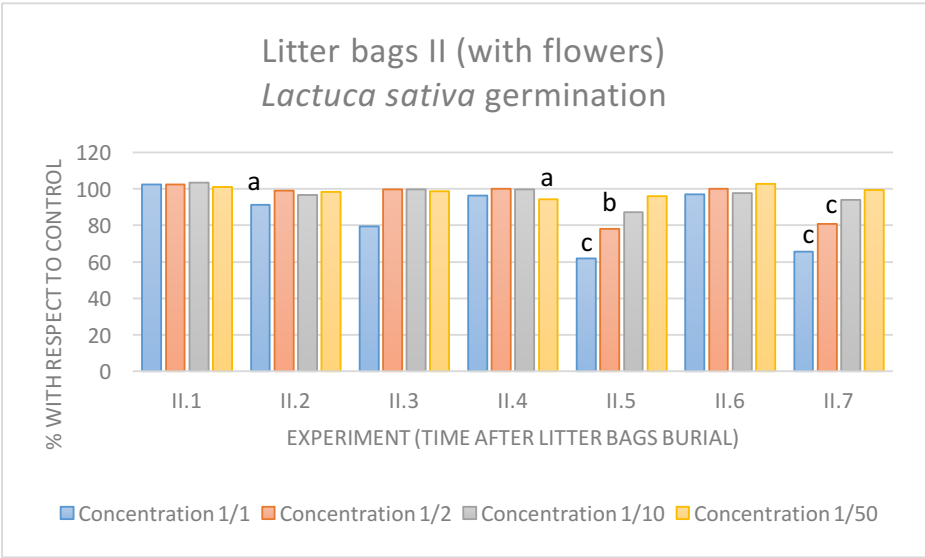


Figure 11.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on germination of *Lactuca sativa*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Litter Bags II.
(Experiments including decomposing flowers)

Figure 11 shows the effects of the leachates obtained from the litter of *Acacia dealbata* (including fallen leaves and flowers) buried in litter bags on *Lactuca sativa* germination. All the statistically significant effects are related to germination inhibition, more slightly at the

beginning (7 days after burial) and stronger at the end (30, 45 and especially 90 days after burial). It seems that the release of allelochemicals from flowers is more powerful but is slower when compared to experiment Litter bags I.

Results on *Lolium perenne* confirm this appreciation (figure 12). Some inhibition at the beginning (one day after burial) but stronger inhibitions after 30, 45 and 90 days. When compared to litter bags I experiment, inhibitions are stronger.

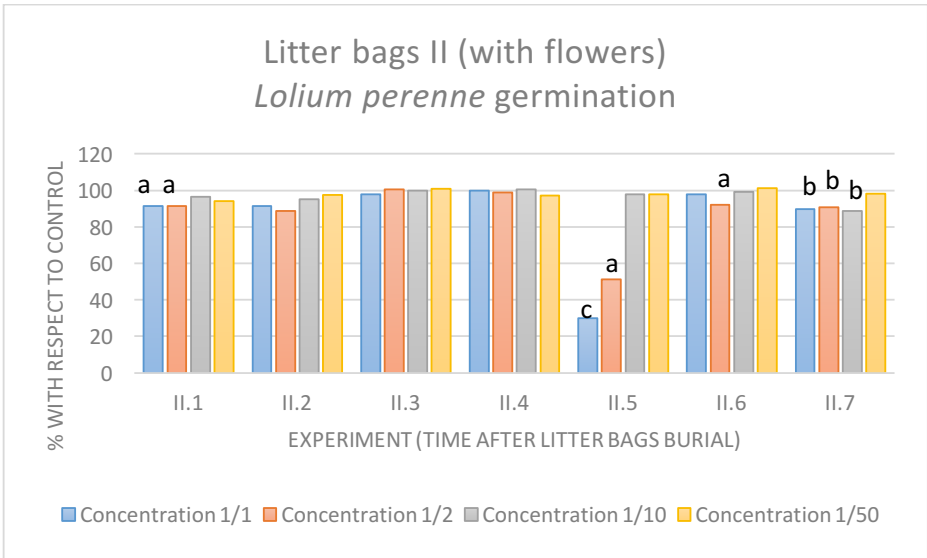


Figure 12.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on germination of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

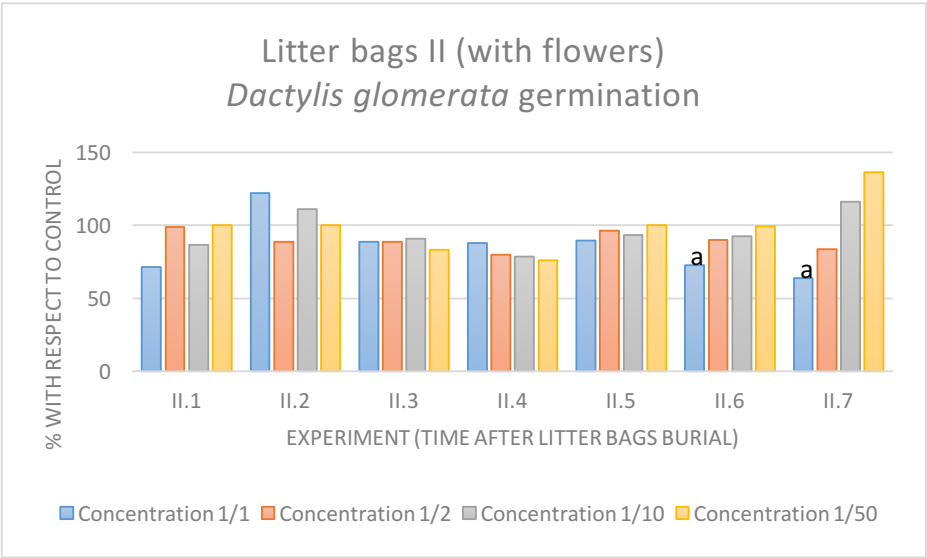


Figure 13.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on germination of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Effects on *Dactylis glomerata* germination (figure 13) are quite similar, but not so marked. Only significant effects are inhibitory, and are located at 45 and 90 days after burial, although in those days, the inhibition is very important.

Figure 14 includes the effects on germination of *Trifolium repens*. The only significant effects are inhibitory and are located at the middle of the experiment (30 days after burial) and at the end (90 days after burial), this confirms the slow release of the allelochemicals by the flowers.

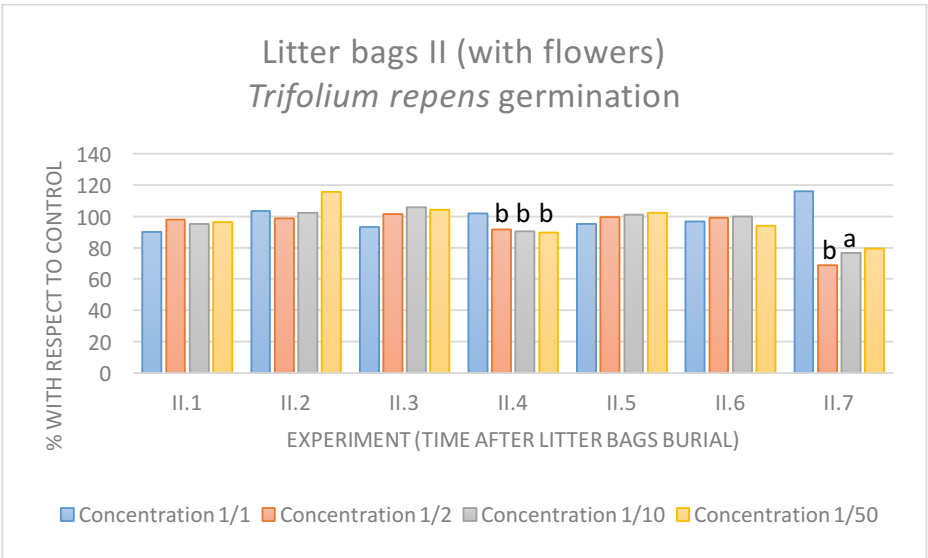


Figure 14.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on germination of *Trifolium repens*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

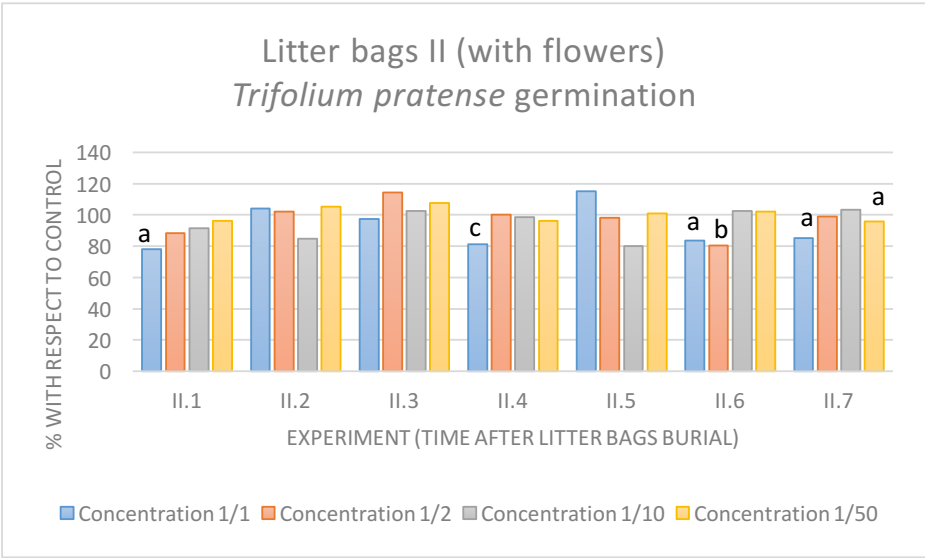


Figure 15.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on germination of *Trifolium pratense*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

In the figure 15 effects on *Trifolium pratense* germination are shown. Here we find more episodes of germination inhibition, with a very fast inhibition one day after burial but more important effects at the end of the series (30, 60 and 90 days after burial). This species was again the most inhibited.

Radicle growth effects on *Lactuca sativa* are shown in figure 16. All significant effects are inhibitory and appear after one week, and more intensively at 30 and 90 days after burial.

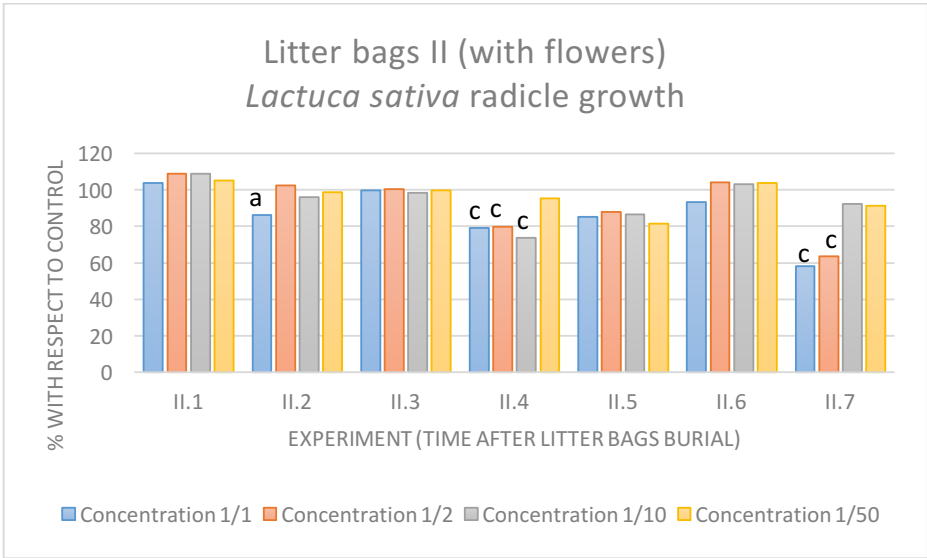


Figure 16.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on radicle growth of *Lactuca sativa*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

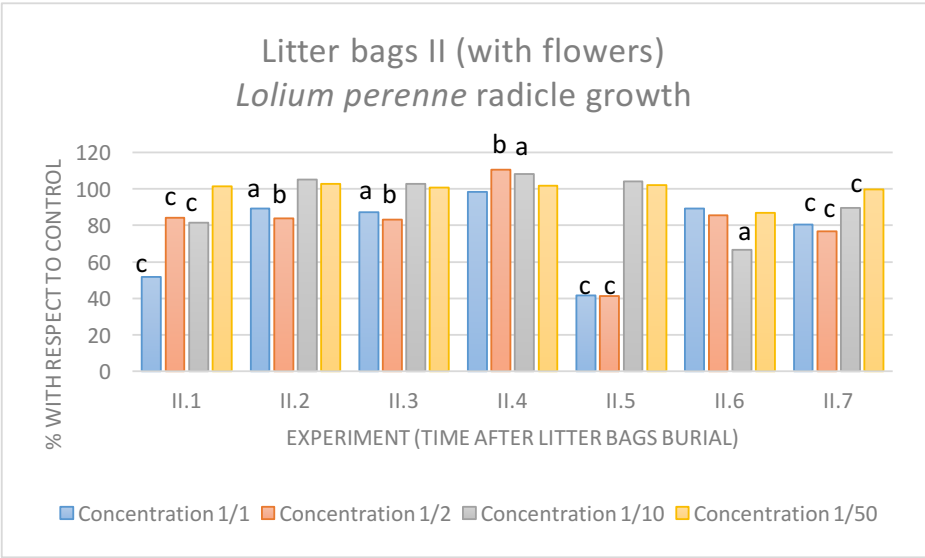


Figure 17.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on radicle growth of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Figure 17 shows the effects on *Lolium perenne*, that although is showing an increase in radicle growth at the middle of the experiment (30 days after burial) shows important inhibition at all the rest of times of assay (1, 7, 15, 45, 60, and 90 days after burial). Considering all the results, this species was highly inhibited when flowers are present. The effects on *Dactylis glomerata* radicle growth are

also interesting, with most of cases inhibited (days 1, 7, 30 and 60 after burial) with only the weakest concentration producing stimulatory effects 7 days after burial.

Both species of *Trifolium* show (figures 19 and 20) some significant stimulatory effects 15 days after burial in both species and additionally after one week in the case of *T. pratense*

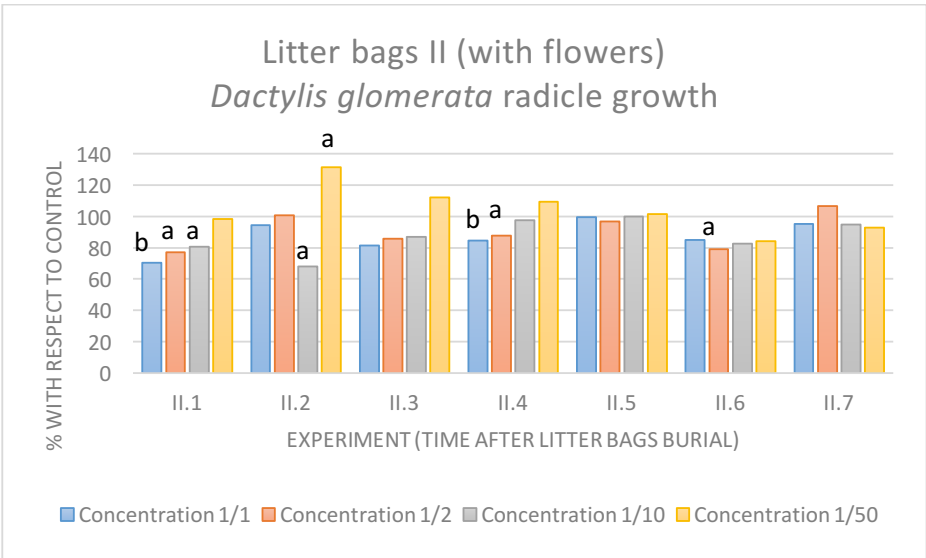


Figure 18.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on radicle growth of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

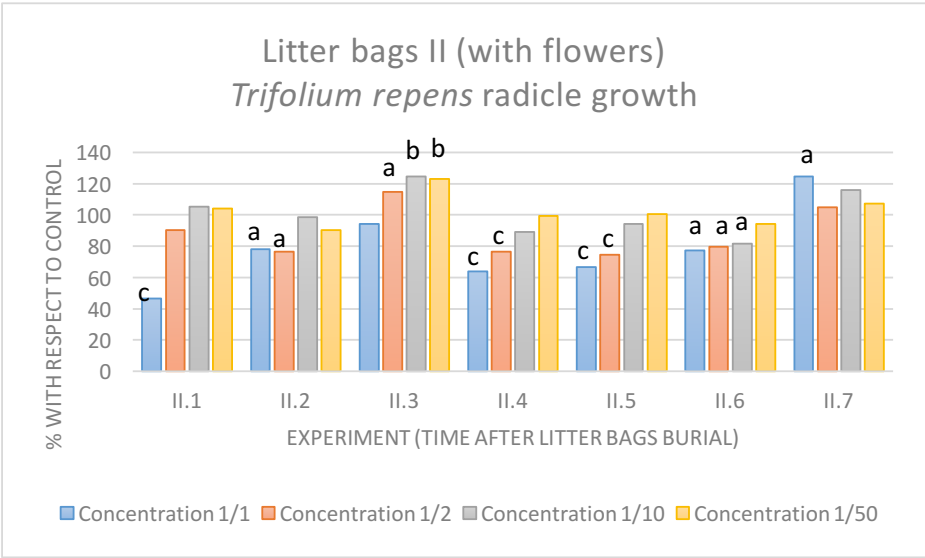


Figure 19.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on radicle growth of *Trifolium repens*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

and after 90 days in the case of *T. repens* with the strongest concentration. But the rest of the series show important inhibitory effects that are produced by several different concentrations, at some times even the most diluted can be very inhibitory.

When analyzing the second series of experiments (Litter bags II), that is, when collected plant materials

included flowers, the results are more marked, showing a fast effect at the beginning and a stronger inhibitory effect at the end of the decomposition process.

This series of experiments show a higher inhibition, probably, because of having a greater proportion of flowers, that in other experiments they already demonstrated to

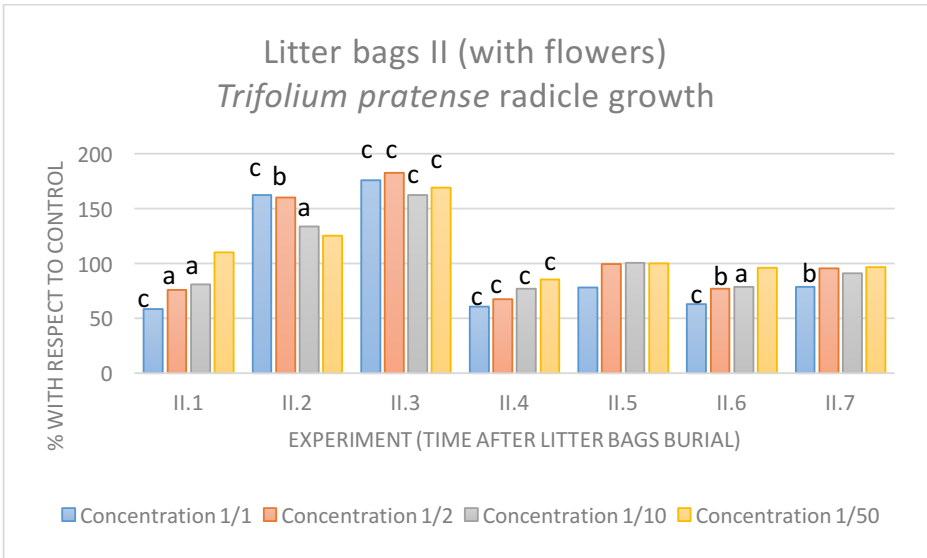


Figure 20.

Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on radicle growth of *Trifolium pratense*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

Table 3.
pH and conductivity values of the extracts in the experiments Litter Bags I.
Electrical conductivity is expressed in $\mu\text{S cm}^{-1}$

pH	I.1	I.2	I.3	I.4	I.5	I.6	I.7
Control	5.4	5.4	5.4	4.6	4.1	3.9	6.1
1/1	6.1	5.2	6.0	6.3	6.1	5.4	6.6
1/2	6.0	5.3	6.0	5.9	5.9	5.4	6.9
1/10	5.9	5.2	6.3	5.1	5.5	5.1	6.9
1/50	5.8	5.4	6.4	4.9	4.9	4.9	6.4
Conductivity							
Control	0	0	0	0	0	0	0
1/1	275	240	330	220	220	165	170
1/2	140	120	170	110	50	70	60
1/10	20	40	10	10	10	10	10
1/50	0	10	0	5	0	5	0

Table 4.
pH and conductivity values of the extracts in the experiments Litter Bags II.
Electrical conductivity is expressed in $\mu\text{S cm}^{-1}$

pH	II.1	II.2	II.3	II.4	II.5	II.6	II.7
Control	6.1	6.2	6.3	6.1	6.3	6.5	6.5
1/1	6.5	7.4	7.8	8.1	7.8	7.7	7.6
1/2	6.8	7.7	8.0	8.2	8.2	8.0	7.6
1/10	7.3	7.8	8.1	8.1	7.6	7.4	7.6
1/50	7.5	7.7	7.7	7.5	7.6	7.4	7.6
Conductivity							
Control	0	0	0	0	0	0	0
1/1	1320	920	700	195	260	235	370
1/2	590	440	300	90	160	115	220
1/10	100	100	50	25	30	10	30
1/50	10	10	0	0	0	0	10

have a great allelopathic capacity (Carballeira and Reigosa, 1999; Casal *et al.*, 1985 Reigosa *et al.*, 1984, 2017a).

Considering all other sources of allelochemicals release studied in other previously published materials, like natural leachates (Reigosa and Carballeira, 1999), decomposition of plant materials mixed in the soil (Reigosa and Carballeira, 2017a) or naturally released allelochemicals by topsoils (Reigosa and Carballeira 2017b) the results of plant litter decomposing in the soil are less marked, although these demonstrated effects could be accumulative, thus explaining the scarcity of species in the underwood of *Acacia dealbata*, combined with the more classical effect of lack of light, nutrients or wate.

It is confirmed that the phytotoxicity is less evident

that when vegetal residues are decomposed added to soil in the laboratory, probably due to the action of the microorganisms (Lorenzo *et al.*, 2011; Kaminsky, 1981; Reigosa and Carballeira, 2017a, 2017b).

The used methodology allows recognizing a real allelopathic phenomenon produced by vegetal residues decomposition of *A .dealbata* in the soil, that will point out, preferably, when the dilution will be scarce and when flowers decompose in the soil. In front of other studies of laboratory it seems that methods of field result more reliable, toxicities result less high and a lot of factors that in the laboratory go unnoticed, here are considered, likewise concentration of allelopathic agents result more suitable to the real ones (Lorenzo *et al.*, 2012; Reigosa, 1988; Stowe, 1979; Stowe and Kil, 1981).

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