



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.

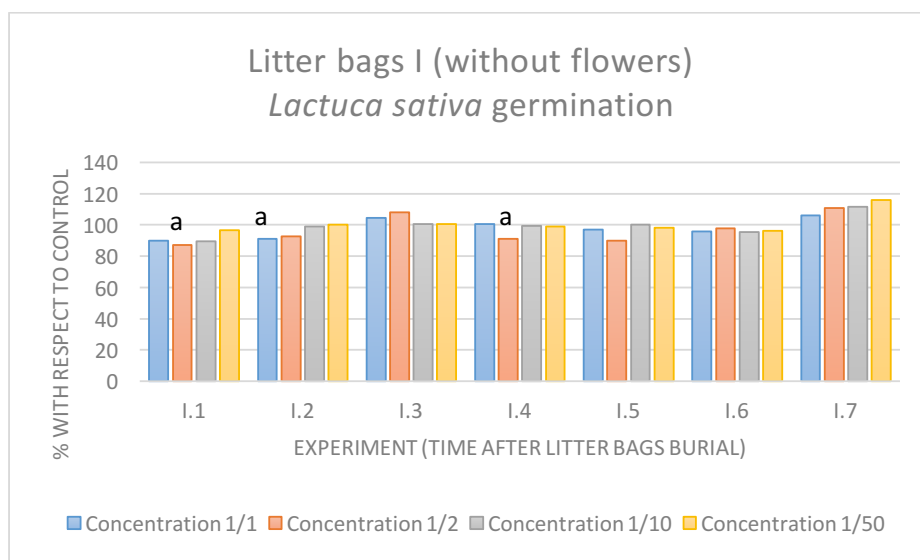


Figure 1.

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).

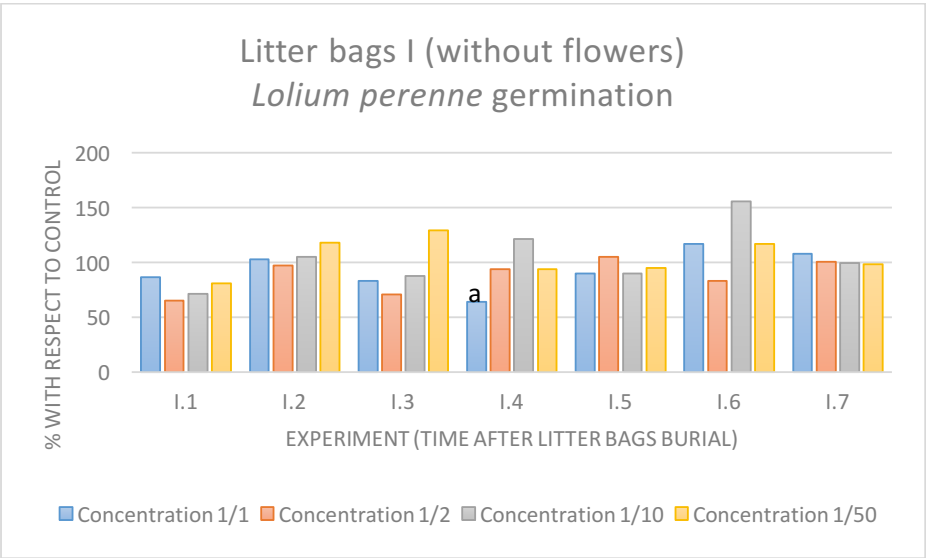


Figure 2.

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.

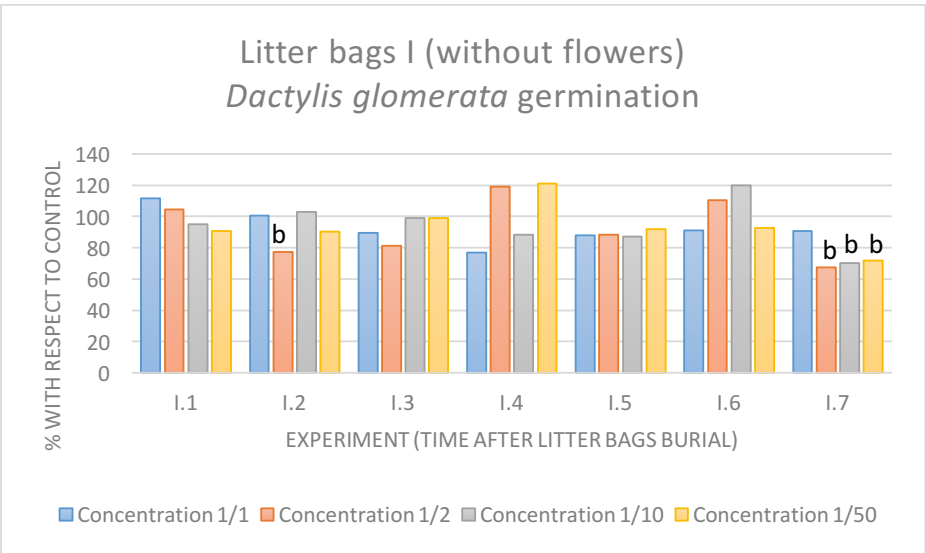


Figure 3.

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).

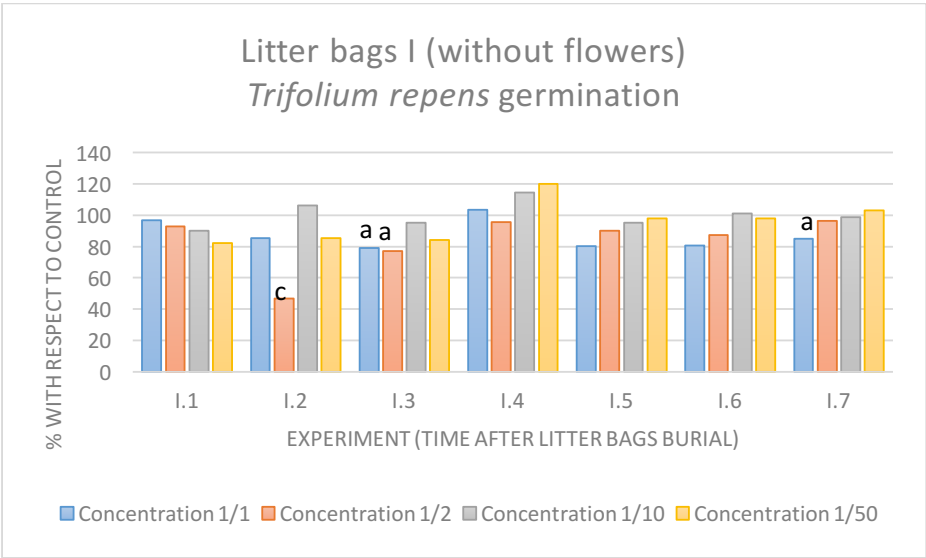


Figure 4.

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.

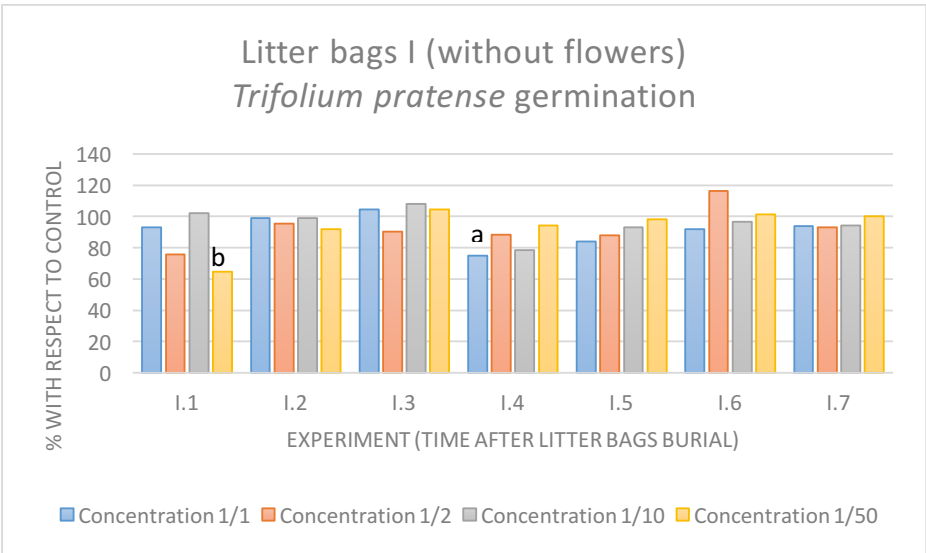


Figure 5.

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).

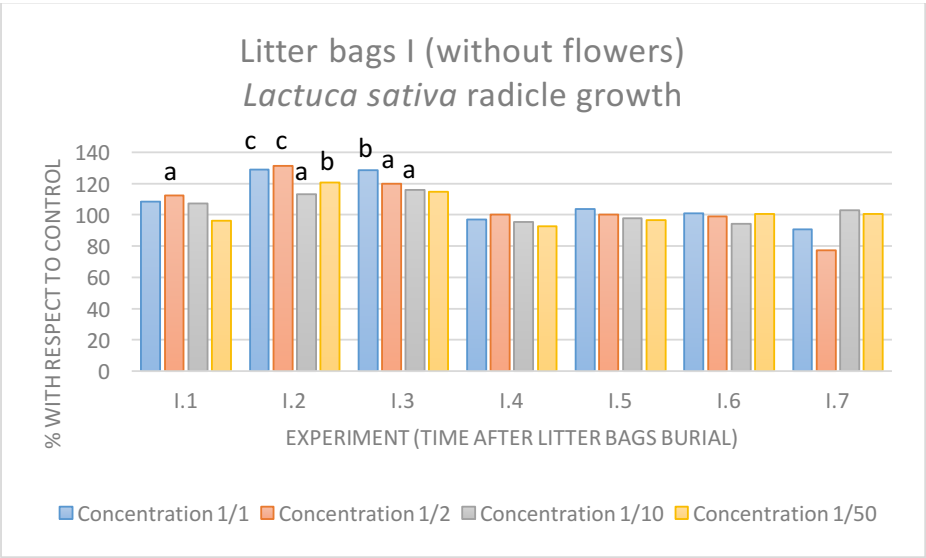


Figure 6. Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Lactuca sativa*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

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).

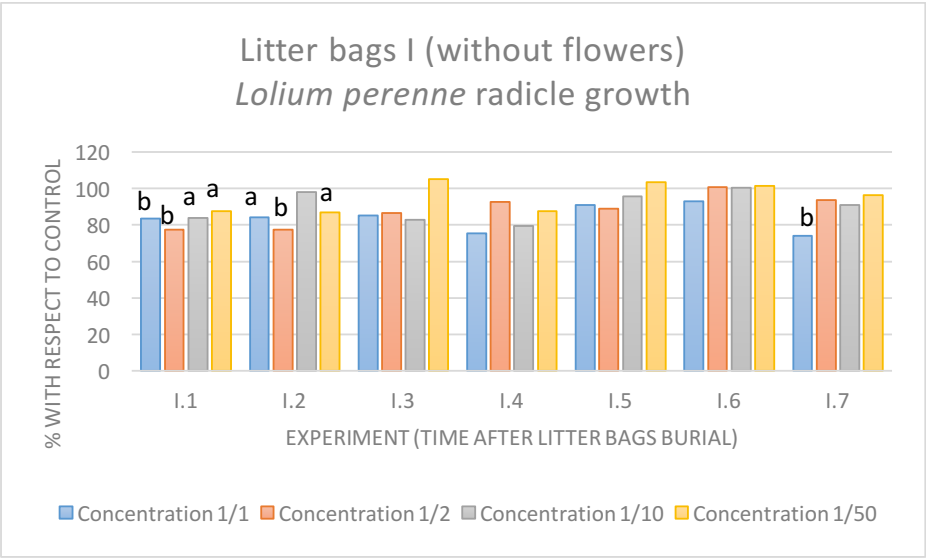


Figure 7. Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment I, litter not containing flowers. Effects on radicle growth of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

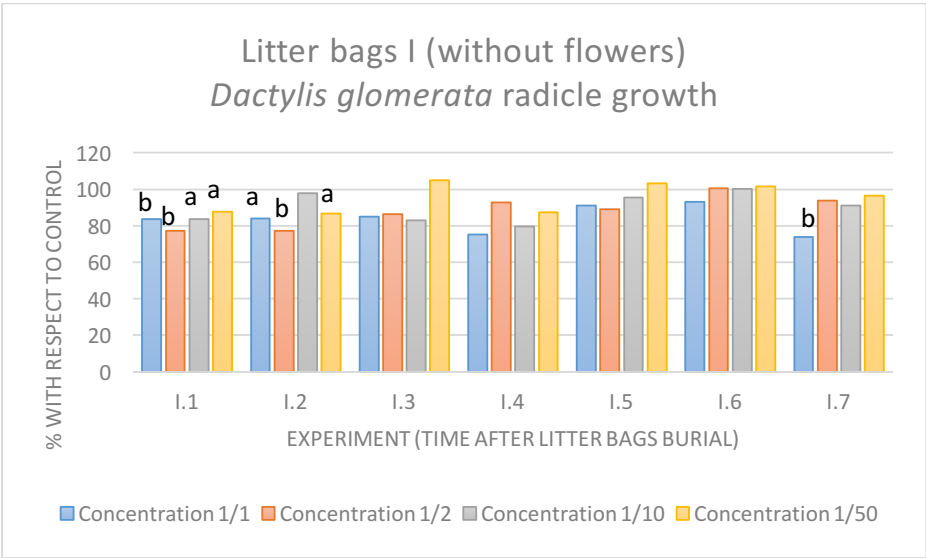


Figure 8.

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.

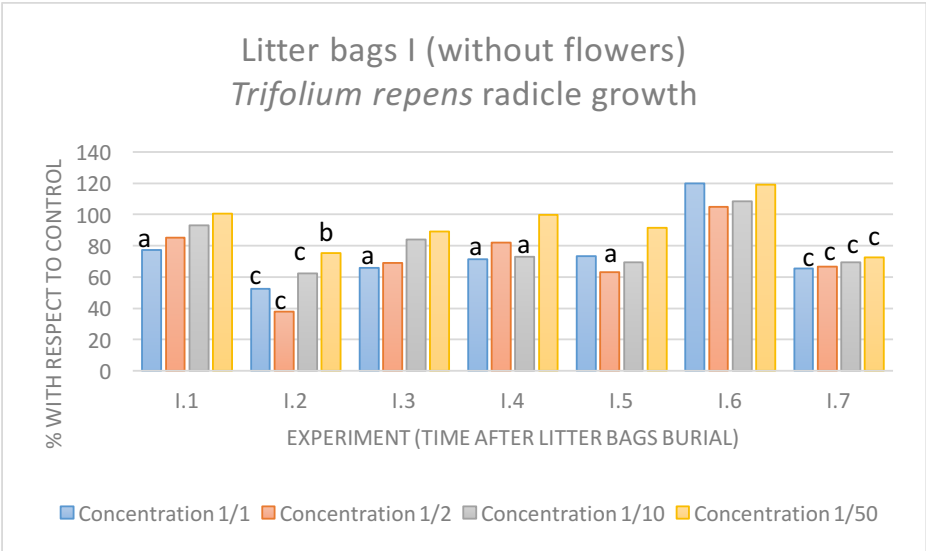


Figure 9.

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).

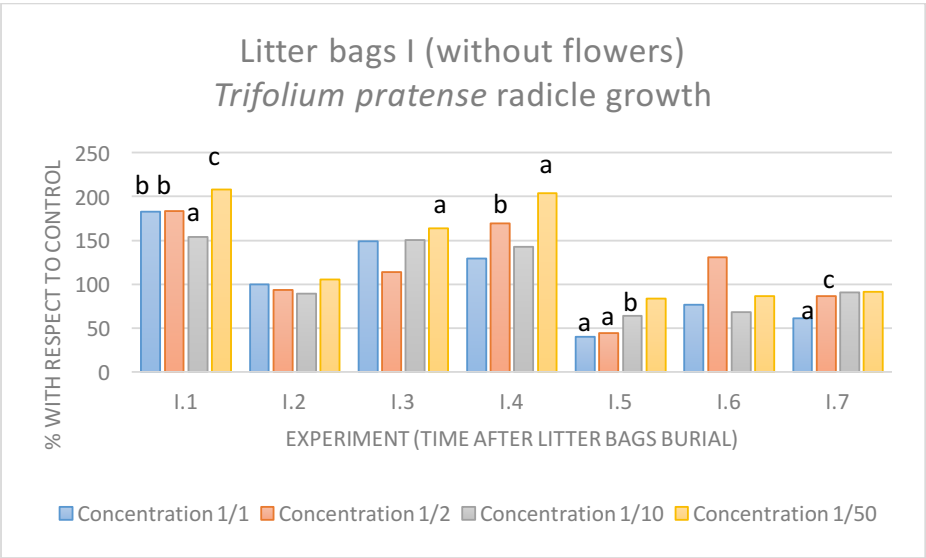


Figure 10.

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

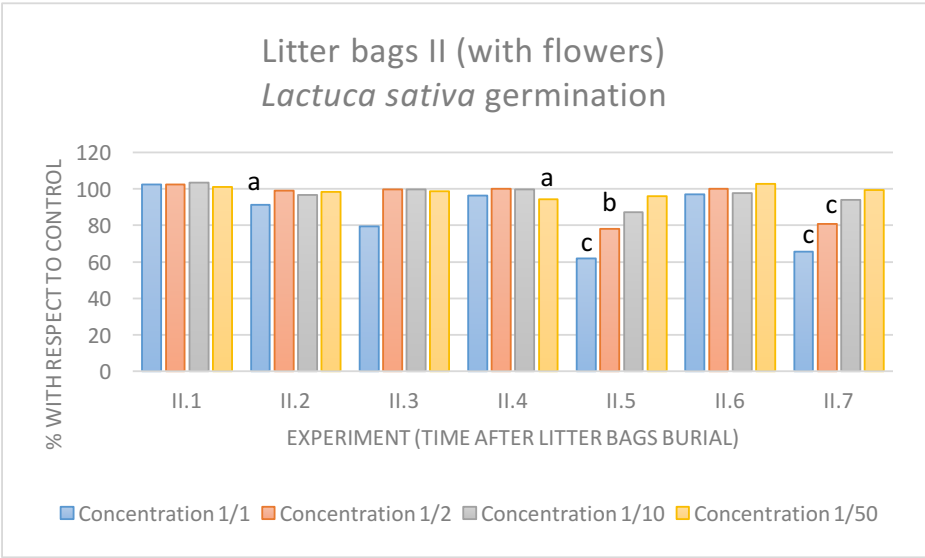


Figure 11.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on germination of *Lactuca sativa*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

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.

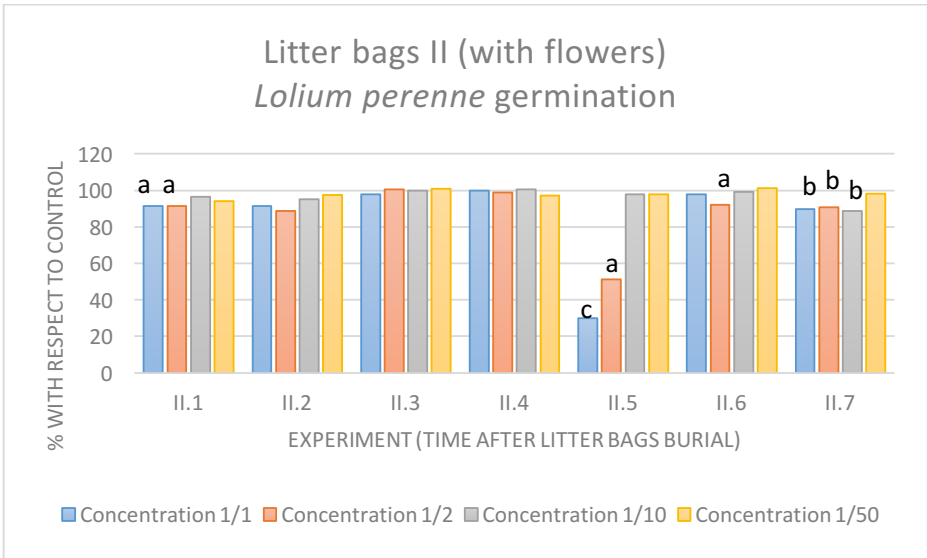


Figure 12.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on germination of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

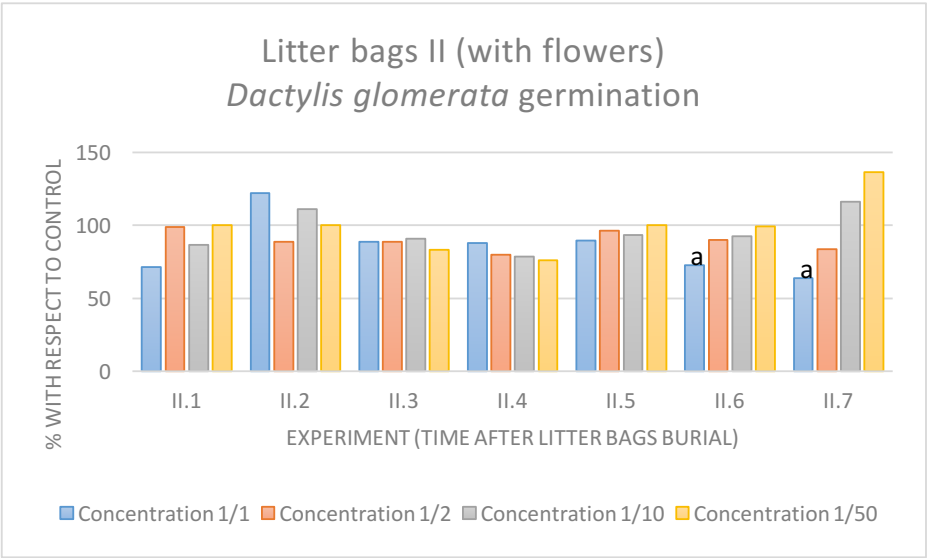


Figure 13.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on germination of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

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.

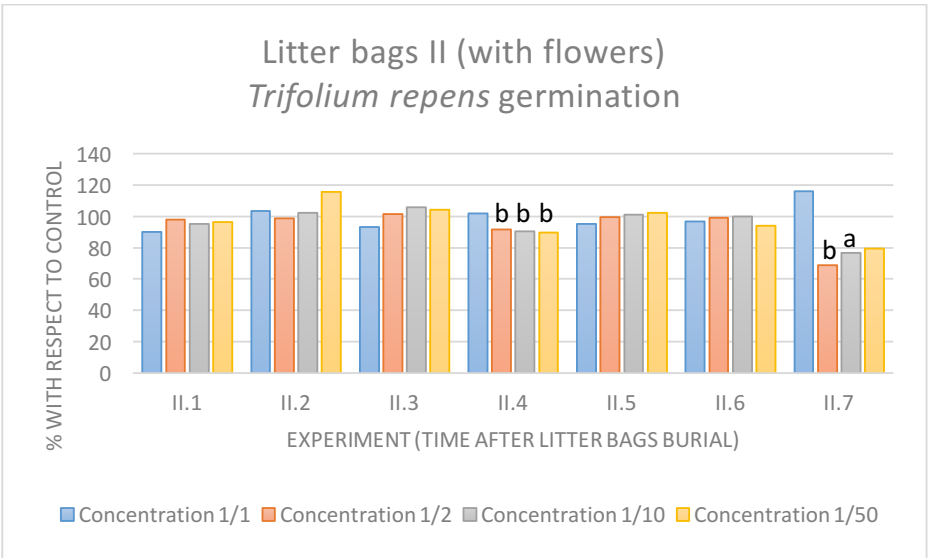


Figure 14.
Effect of leachates obtained washing litter decomposed in situ in plastic bags. Experiment II, litter containing flowers. Effects on germination of *Trifolium repens*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

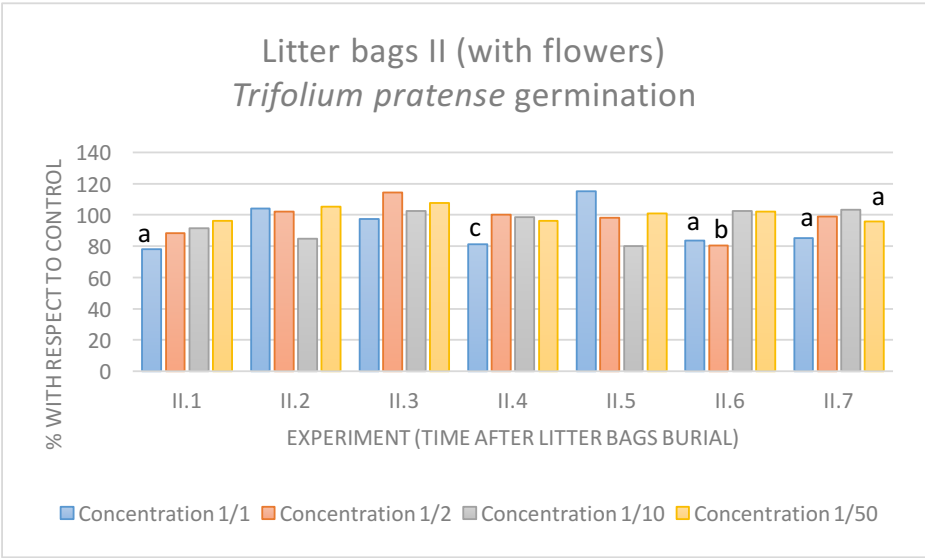


Figure 15.

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.

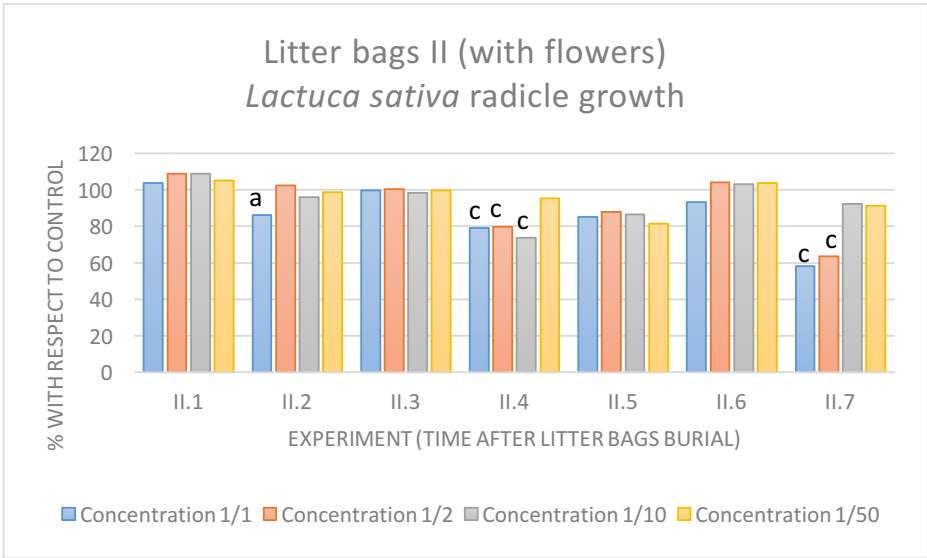


Figure 16.

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).

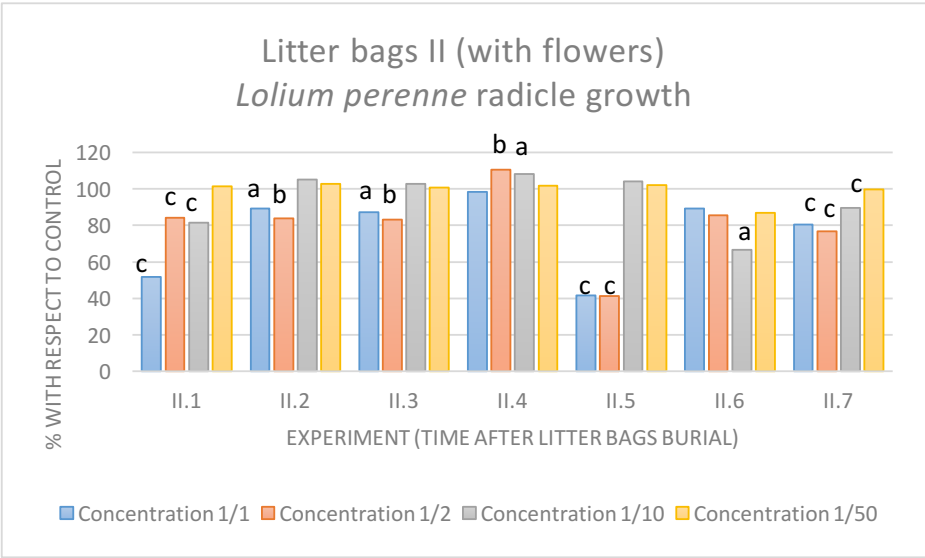


Figure 17.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on radicle growth of *Lolium perenne*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

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*

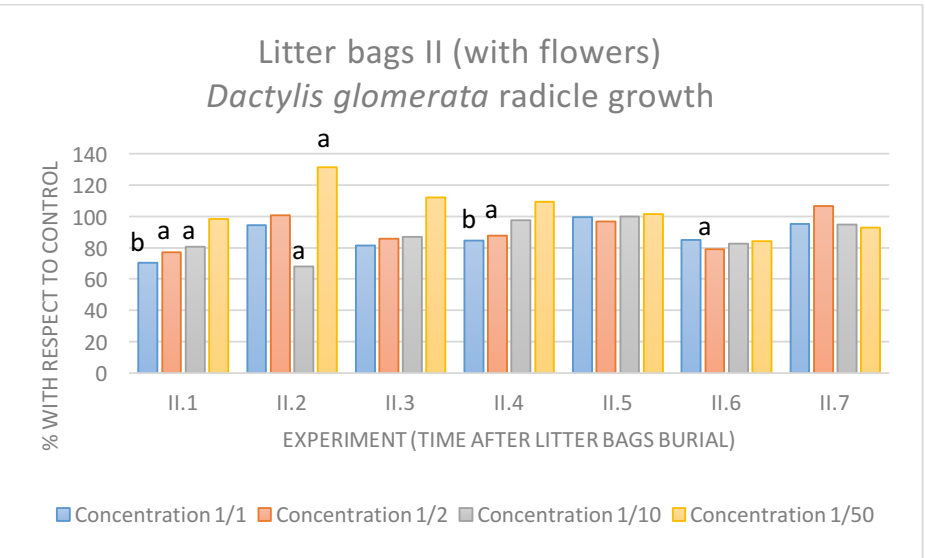


Figure 18.
Effect of leachates obtained washing litter decomposed in situ in plastic bags.
Experiment II, litter containing flowers. Effects on radicle growth of *Dactylis glomerata*. a, b, c, significant $p<0.001$, significant $p<0.01$, significant $p<0.05$ respectively (LSD test).

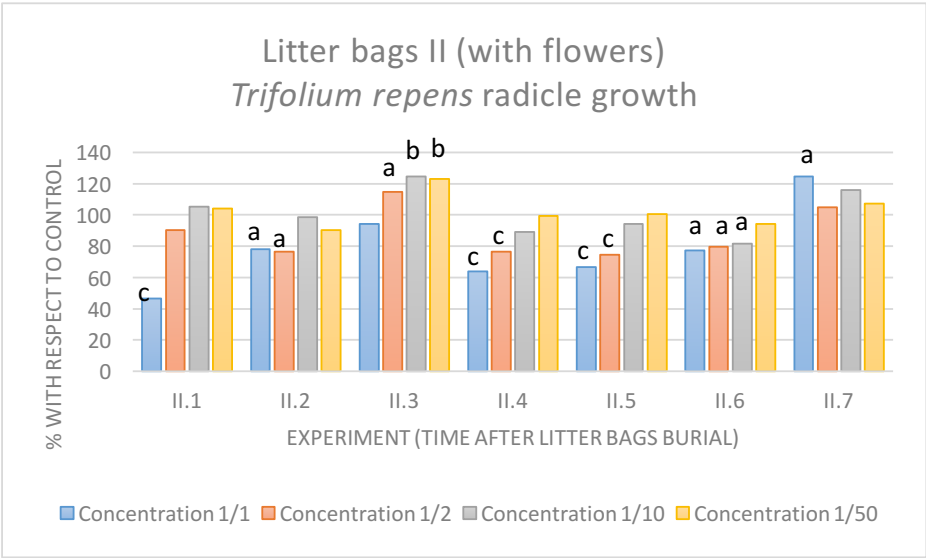


Figure 19.

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

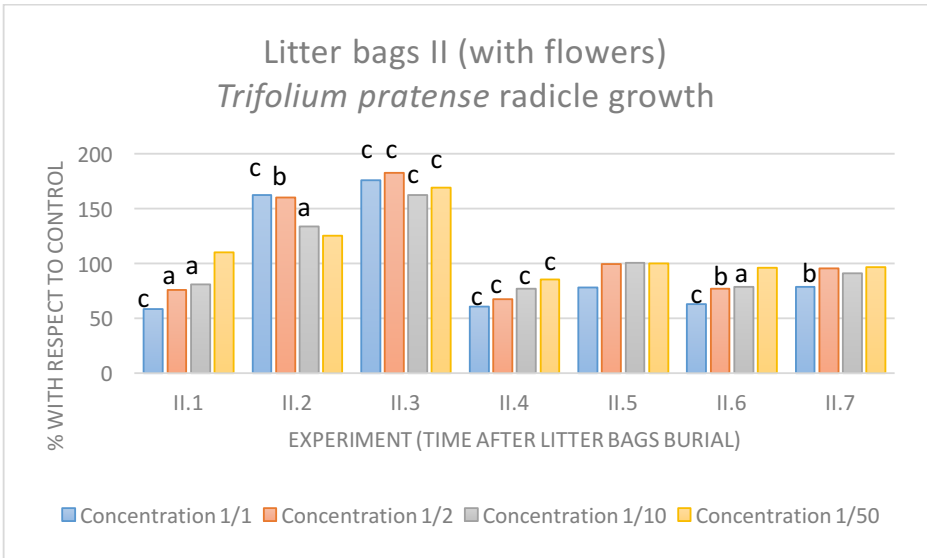


Figure 20.

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).

References

- Aguilera N., Becerra J., Villasenor-Parada C., Lorenzo P., González L., Hernandez V. (2015) Effects and identification of chemical compounds released from the invasive *Acacia dealbata* Link. Chemistry and Ecology 31(6): 479-493. DOI: 10.1080/02757540.2015.1050004
- Araniti F., Gulli T., Marrelli M., Statti G., Gelsomino A., Abenavoli M.R. (2016) *Artemisia arborescens* L. leaf litter: phytotoxic activity and phytochemical characterization. Acta Physiologiae Plantarum 38(5) 128. DOI: 10.1007/s11738-016-2141-7
- Aslam F., Khaliq A., Matloob A., Tanveer A., Hussain, S., Zahir Z. (2017) Allelopathy in agro-ecosystems: a critical review of wheat allelopathy-concepts and implications. Chemoecology 27(1): 1-24. DOI: 10.1007/s00049-016-0225-x
- Attiwill P.M., Guthrie H.B., Leuning (1978). Nutrient cycling in a *Eucalyptus obliqua* (L'Herit) Forest. I. Litter production and nutrient return. Aust. J. Bot. 26: 79-91.
- Ballester A. (1971). Estudio del contenido de sustancias de crecimiento e inhibidores de la germinación de *Erica cinerea*. Ph. D. thesis, Univ. Santiago de Compostela.
- Ballester A. (1972). Alelopatía: Interacción química entre especies vegetales. Acta Científica Compostelana 9 (3-4): 145-151.
- Bauer J.T., Shannon S.M., Stoops R.E., Reynolds H.L. (2012) Context dependency of the allelopathic effects of *Lonicera maackii* on seed germination. Plant Ecology 213(12): 1907-1916.
- Blum U., Gerig T.M., Worsham A.D., King L.D. (1993). Modification of Allelopathic Effects of p-Coumaric Acid on Morning-Glory Seedling Biomass by Glucose, Methionine, and Nitrate. J. Chem. Ecol. 19 (12): 2791-2811.
- Blum U., Worsham A.D., King L.D., Gerig T.M. (1994). Use of water and EDTA extractions to estimate available (free and reversibly bound) phenolic acids in cecil soils. J. Chem. Ecol. 20 (2): 341-359.
- Bocock K.L. (1964). Changes in the amount of dry matter, nitrogen, carbon and energy in decomposing woodland leaf litter in relation to the activities of the soil fauna. J. Ecol. 52: 273-284.
- Carballeira A., Cuervo A. (1980). Seasonal Variation in Allelopathic Potential of Soils from *Erica australis* L. Heathland. Acta Oecologica. Oecol. Plant. 1 (15), 4: 345-353.
- Casal J.F., Reigosa M.J., Carballeira A. (1985). Potentiel allélopathique de *Acacia dealbata* Link. Rev. Ecol. Biol. Sol 2 (1): 1-12.
- Chaves N., Alias J.C., Sosa T. (2016) Phytotoxicity of *Cistus ladanifer* L.: Role of Allelopathy. Allelopathy Journal 38(2): 113-131.
- Chou C.H. (2010) Role of allelopathy in sustainable agriculture: Use of allelochemicals as naturally occurring bio-agrochemicals. Allelopathy Journal 25(1): 3-16.
- Dastagir G., Hussain F. (2015) Allelopathic Potential of *Quercus baloot* Griff. Pakistan Journal of Botany 47(6): 2409-2414.
- Dawkins K., Esiobu N. (2016) Emerging Insights on Brazilian Pepper Tree (*Schinus terebinthifolius*) Invasion: The Potential Role of Soil Microorganisms. Frontiers in Plant Science 7-712.
- Del Moral R., Cates R.G. (1971). Allelopathic Potential of the Dominant Vegetation of Western Washington. Ecology 52 (6): 1030-1037.
- Del Moral R., Muller C.H. (1969). Fog Drip: A Mechanism of Toxin Transport from *Eucalyptus globulus*. Bull. Torrey Bot. Club 96: 467-475.
- Gloaguen J.C., Touffet J. (1980). Vitesse de décomposition et évolution minérale des litières sous climat atlantique. I. Le Hêtre et quelques conifères. Acta Oecologica Oecol. Plant. 15: 3-26.
- Goel U., Saxena D.B., Gupta A.K. (1994). Allelopathic potential of *Chenopodium album* L. Allelopathy Journal 1 (2): 105-113.
- Grant W.D. (1976). Microbial Degradation of Condensed Tannins. Science 193: 1137-1138.
- Guenzi W., McCalla T.M. (1966). Phytotoxic Substances Extracted from Soil. Soil Sci. Soc. Amer. Proc. 30: 214-216.
- Halvorson J.J., Belesky D.P., West M.S. (2017) Inhibition of forage seed germination by leaf litter extracts of overstory hardwoods used in silvopastoral systems. Agroforestry Systems 91(1): 69-83.
- Inderjit, Dakshini K.M.M. (1994). Effect of Cultivation on Allelopathic Interference Success of the Weed, *Pluchea lanceolata*. J. Chem. Ecol. 20 (5): 1179-1188.
- Jalal M.A.F., Read D.J. (1983). The Organic Acid Composition of *Calluna* Heathland Soil with Special Reference to Phyto and Fungitoxicity. II. Monthly Quantitative Determination of the Organic Acid Content of *Calluna* and Spruce Dominated Soils. Pl. and Soil 70: 273-286.
- Juste J., Reigosa M.J., Carballeira A. (1985). Circuitos de liberación de agentes aleloquímicos en *Rumex obtusifolius* L. Trabajos Compostelanos de Biología 12: 205-213.
- Kaminsaky R. (1981). The Microbial Origin of the Allelopathic Potential of *Adenostoma fasciculatum*. H. and A. Ecol. Monogr. 5 (3): 365-382.
- Kaur S., Singh H.P., Batish D.R., Kohli R.K. (2012) Phytotoxicity of decomposing below-ground residues of *Ageratum conyzoides*: nature and dynamics of release of phytotoxins. Acta Physiol Plant 34:1075-1081. DOI 10.1007/s11738-011-0904-8
- Kunc F. (1971). Decomposition of Vanillin by Soil Microorganisms. Folia Microbiol. (Prague) 16: 41-50.
- Leon W.B. (1976). Phytotoxicité induite par les résidus de récolte de *Sorghum vulgare* dans les sols sableux de l'ouest Africain. Th. Doc. Univ. Nancy, France.
- Li J.Q., Fergola P., Ma Z. (2011) Effects of allelochemicals

- produced by one population in a chemostat-like environment. *Journal of Theoretical Biology* 284(1): 99-105.
- Lodhi M.A.K. (1975). Soil-plant Phytotoxicity and its Possible Significance in Patterning of Herbaceous Vegetation in a Bottomland Forest. *Amer. J. Bot.* 62: 618-622.
- Lodhi M.A.K. (1976). Role of Allelopathy as Expressed by Dominating Trees in a Lowland Forest in Controlling Productivity and Pattern of Herbaceous Growth. *Amer. J. Bot.* 63: 1-8.
- Lorenzo, P. Pazos-Malvido, E., González, L. and Reigosa, M.J. (2008). Allelopathic Interference of Invasive *Acacia dealbata*: Physiological Effects. *Allelopathy Journal* 22(2): 452-462.
- Lorenzo P, González L, y Reigosa MJ (2010) The genus *Acacia* as invader: the characteristic case of *Acacia dealbata* Link in Europe. *Annals of Forest Science.* 67(1): 101. DOI: 10.1051/forest/2009082.
- Lorenzo, P., Palomera-Pérez, A., Reigosa, M.J., González, L. (2011). Allelopathic interference of invasive *Acacia dealbata* Link on the physiological parameters of native understory species. *Plant Ecology* 212 (3): 403-412. DOI:10.1007/s11258-010-9831-9.
- Lorenzo, Paula; Pazos-Malvido, Eva; Rubido-Bara, Margarita; Reigosa, M.J.; González, L. (2012). Invasion by the leguminous tree *Acacia dealbata* (Mimosaceae) reduces the native understorey plant species in different communities. *Australian Journal of Botany* 60(8): 669-675
- Macauley B.J. (1975). Biodegradation of litter in *Eucalyptus pauciflora* communities. I. Techniques for comparing the effects of fungi and insects. *Soil Biol. Biochem.* 7: 341-344.
- Mitchell C.A. (1968). Detection of carbohydrates leached from aboveground plant parts. M. S. Thesis, Cornell Univ., Ithaca.
- Molina A., Reigosa M.J., Carballeira A. (1984). Efectos alelopáticos durante la descomposición de residuos de *Eucalyptus globulus* Labill. *Publ. Seminario Estudos Galegos* 5: 117-131.
- Muller C.H., Chou C.H. (1972). Phytotoxins: An Ecological Phase of Phytochemistry. In: *Phytochemical Ecology*, p. 201-216, J. B. Harborne (ed.), Academic Press, New York.
- Nilsson M.C., Gallet C., Wallstedt A. (1998) Temporal variability of phenolics and batatasin-III in *Empetrum hermaphroditum* leaves over an eight-year period: interpretations of ecological function. *OIKOS* 81(1): 6-16.
- Patrick Z.A. (1971). Phytotoxic Substances Associated with the Decomposition in Soil of Plant Residues. *Soil Sci.* 111: 13-18.
- Patrick Z.A., Koch L.W. (1958). Inhibition of Respiration, Germination and Growth by Substances Arising During the Decomposition of Certain Plant Residues in the Soil. *Can. J. Bot.* 36: 621-647.
- Quarterman E. (1973). Allelopathy in Cedar Glades Plant Communities. *J. Tennessee Acad. Sci.* 48: 147-150.
- Reigosa M. J. (1988). Estudio del potencial alelopático de *Acacia dealbata* Link. Ph. D. Thesis, Univ. Santiago de Compostela, Serv. Publ. Univ. Santiago de Compostela.
- Reigosa MJ, Carballeira A (1999) Effects of natural leachates of *Acacia dealbata* Link in Galicia (NW Spain). *Botanical Bulletin of Academia Sinica* 40: 87-92.
- Reigosa, M.J.; Carballeira, A. (2017a) Studies on the Allelopathic Potential of *Acacia dealbata* Link.: Allelopathic Potential Produced During Laboratory Decomposition of Plant Residues Incorporated into Soil. *Journal of Allelochemical Interactions* 3(1): 23-33.
- Reigosa, M.J.; Carballeira, A. (2017b) Phytotoxicity in Topsoils Collected under *Acacia dealbata* Link in Galicia (NW Spain). *Journal of Allelochemical Interactions* 3(1): 15-21
- Reigosa M.J., Casal J.F., Carballeira A. (1984). Efectos alelopáticos de *Acacia dealbata* Link durante su floración. *Studia Oecologica*, V: 135-150.
- Rice E.L. (1984). Allelopathy. Academic Press.
- Robinson R.K. (1971). Importance of Soil Toxicity in Relation to Stability of Plant Communities. In: *The Scientific Management of Animal and Plant Communities for Conservation*. Eds. E. Duffey and A. S. Watt. pp. 105-113.
- Rovira A.D. (1969). Plant root exudates. *Bot. Rev.* 35: 35-39.
- Sokhal R.R., Rohlf F.J. (1979). *Biometría. Principios y métodos estadísticos en la investigación biológica*. Ed. H. Blume.
- Squires V.R., Trollope W.S.W. (1978). Allelopathy in the Karoo Shrub *Chrysocoma tenuifolia*. *S. African Sc.* 75: 85-89.
- Stevenson F.J. (1967). Organic Acids in Soil. In: *Biochemistry*, pp. 119-142. Marcel Dekker, Inc., n. 4.
- Stowe L.G. (1979). Allelopathy and its influence on the distribution of plants in an Illinois old-field. *J. Ecol.* 67: 1065-1085.
- Stowe L.G., Kil B.-S. (1981) The role of toxins in plant-plant interactions. *Handbook of natural toxins*, vol. 1, Plant and Fungal Toxins 707-741.
- Tack B.F., Chapman P.J., Dagley S. (1972). Metabolism of Gallic and Syringic Acids by *Pseudomonas putida*. *J. Biol. Chem.* 247: 6438-6433.
- Topuzovic M.D., Radojevic I.D., Dekic M.S., Radulovic N.S., Vasic S.M., Comic L.R., Ljiljana R., Licina B.Z. (2015) Phytomedical investigation of *Najas minor* All. In the view of the chemical constituents. *EXCLI JOURNAL* 14: 496-503.
- Tukey H.B. Jr. (1966). Leaching of Metabolites from Above-Ground Plant Parts and its Implications. *Bull Torrey Bot. Club* 93: 385-401.
- Tukey H.B. Jr. (1969). Implications of allelopathy in agricultural plant science. *Bot. Rev.* 35: 1-16.

- Turner J. A., Rice E.L. (1975). Microbial Decomposition of Ferulic Acid in Soil. J. Chem. Ecol. 1: 41-58.
- Wang R.L., Kang X.W., Quan G.M., Zhang J.E. (2015) Influence of *Lantana camara* on soil II. Effects of *Lantana camara* leaf litter on plants and soil properties. Allelopathy Journal 35(2): 207-215.
- Wei W., Zhou M., Xian X.Y., Zheng C.X., Hou Y.P. (2017) Effects of aqueous root leachates from dominant tree species on seed germination and seedling growth of exotic plant *Rhus typhina* L. in Shandong Peninsula. Allelopathy Journal 40(1): 71-80.
- Whitehead D.C. (1964). Identification of p-Hydroxybenzoic, Vanillic, p-Coumaric and Ferulic Acids in Soils. Nature,
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