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Effects of natural leachates of *Acacia dealbata* Link in Galicia (NW Spain)

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Abstract. A systematic collection of throughfall (rain passing through the canopies), stemflow (rain flowing over the stems), and soil percolates was carried out in a *Acacia dealbata* plantation during one year, and its allelopathic capacity was biotested using *Lactuca sativa* L. var. Great Lakes. The results showed an allelopathic effect related to flowers: Germination of *L. sativa* was inhibited 30% by throughfall, 60% by stemflow, and 75% by soil percolates during the blossoming of *A. dealbata*, while radicle growth of *L. sativa* was inhibited 23%, 33%, and 48% by the same solutions. Climatic factors, particularly rainfall some days before, were also important. The three types of samples showed significant toxicities during some periods of the year, particularly percolates, which retained toxicity for a longer time, due perhaps to the decomposition of flowers fallen on the ground.

Keywords: *Acacia dealbata*; Allelopathy; Percolate; Stemflow; Throughfall.

Introduction

Certain allelopathic phenomena result when toxins leach into rainfall or dew (Carral et al., 1988; Del Moral and Muller, 1969; Juste et al., 1985; Molina et al., 1991; Rama Devi et al., 1997). Demonstration of allelopathy generally has been accomplished through biotests “made in laboratory” (Hibbs and Schumaker, 1987; Leather and Einhellig, 1987; Rice, 1984). The validity of some of these tests is debatable due to the artificial manner in which the solutions of the supposed allelopathic agents were obtained (Harper, 1977; Inderjit and Dakshini, 1995; Stowe and Kil, 1981; Wardle, 1987). It is often difficult in some bioassays reported in the literature to know if the concentrations of allelochemicals tested are ecologically meaningful. There is often a lack of correlation between artificial assays and field experimental data (Henn et al., 1988; Harper, 1977; Stowe, 1979).

Some species of the genus *Acacia* have shown phytotoxic capacity (Duham and Lakshminarayana, 1995; Elakovich and Wooten, 1995; González et al., 1995; Nsolomo et al., 1995; Puri et al., 1994; Saxena and Sharma, 1996; Souto et al., 1994, 1995).

Acacia dealbata Link is a leguminous plant coming from Australia and Tasmania. Introduced in the European continent in 1820, it is very common in Mediterranean countries where it is both found in the wild and farmed for ornamental use. *Acacia dealbata* can become a blight (García, 1979). It invades vineyards, grows in meadows, destroys retaining walls, pulls archaeological excavations down and makes access to natural areas difficult. In addition to its great colonizing capacity it leads to a very low covering and scarcity of undergrowth species. Based on this, the existence of allelopathy was first suspected. *A priori*, the probability of such hypothesis was supported by the fact that, as Rabotnov (1974) said, “allelopathic phenomena are chiefly possible between species originating in different regions.”

A later series of experiments (Casal et al., 1985; Reigosa et al., 1984) showed *A. dealbata* to have a great potential allelopathic capacity through several mechanisms fluctuating within the year and within short periods of time. These fluctuations could have important ecological consequences if the highest moments of toxin production coincided with the most critical period for the receiver (Muller, 1970).

Rice (1984) indicated that it is necessary to get more information concerning rates of production and escape to the environment of the allelopathic compounds. In this paper we report that during one year we collected the natural throughfall (water that passes through the canopy and falls to the soil), stemflow (water fraction that arrives to the soil flowing over the stems), and percolates, recording data on rain episodes and nutrient leakage. Our objective was to test the possibility that allelopathic phenomena occurred in response to toxins and also to determine the influence of the phenological phase of the *A. dealbata* and meteorological factors on the allelochemical effects.

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Material and Methods

Area of Study and Characteristics

The area of study is located at 4°53’15” W and 42°52’14” N (Santiago de Compostela) at an altitude of 170 m with a slope of 15% and an orientation of 215° N. It has already been described in detail by Casal et al. (1985). The area is settled on an AC horizons soil, where the rock is granite of middle degree that is very altered, quite rich in biotite, poor in quartz, and with a little muscovite. The soil samples have been collected from an O non-hydromorphe horizon whose thickness fluctuates between 20 and 40 cm.

Climate

Carballeira et al. (1983) classifies the area of study as the climate type “Perhumid-Mesothermal I - without lack of water” as per the classification of Thornwaite, “Cool maritime” as per the one of Papadakis and belonging to the phytoclimatic subregion named “Atlantic European” of Allué. During the period of this study the conditions were similar to the normal ones in the zone, that is: yearly rainfall: 1865 mm, summer rainfall: 202 mm, average yearly temperature: 12.5°C, average maximum temperature in the warmest month and average minimum in the coldest month: 23.5 and 3.1°C respectively. Its pluviometric regime is of the Atlantic type, with well spread rains during all of the seasons (Carballeira et al., 1983).

Vegetation

The dominant species is *Acacia dealbata* with individuals more than 25 years old. The maximum height is 15.10 m; maximum diameter is 24.3 cm at a height of 1.35 m. Its horizontal distribution is made by aggregates at random, with an average density of 11.9 individuals per square meter. The quantitative analysis of the acacia area undergrowth was carried out by establishing seven squares, each with sides of five meters, in the present species covering. The squares were distributed at random and subsampled by using 24 squares with sides of 30 cm. In this way the set of the species (*A. dealbata* excluded) showed a covering lower than 1%, the flowers range inventory being reduced to *Rubus* sp., *Hedera helix* L., *Agrostis curtisii* Kerguélen, *Umbilicus rupestris* (Salisb.) Dandy, *Laccaria laccata* Fr. ex Scop., *Polyporus umbellatus* Peerson ex Fries and *Trametes* sp.

Collection of the Natural Washings

First of all, as a control in order to know the pluviometric characteristics of the area, a plastic tray 100 cm long, 11.5 cm wide, and with a semicircular section at 30 cm height from the ground was connected through a rubber tube to an opaque plastic tank of 4.3 L capacity that was buried in the ground in a field adjacent to the acacia grove where the collections were made. Rainfall was collected, and was interpreted as a mean of the water and nutrients coming to the ecosystem through the atmosphere. Rainfall data were analyzed by a model of simple linear regression. Data were collected from the nearest meteorological station, which was at the Instituto de Investigaciones Agrobiológicas, CSIC, Santiago de Compostela.

Inside the experimental plot, eight rain gauges similar to the control were randomly distributed, in order to collect the throughfall (Molina et al., 1991). In addition stemflow containers connected to both plastic bottles of the same characteristics mentioned above were placed in eight trees chosen at random. Considering the small diameter of the trunks, the collectors were modeled *in situ* with a hot mixture of wax (90%) and resin (10%), as shown in Figure 1. Once cold, they were covered with an impermeable varnish to avoid the leaching of ions into the stemflow; this was tested by using distilled water and analyzing it with the result that none of the studied ions showed any enrichment.

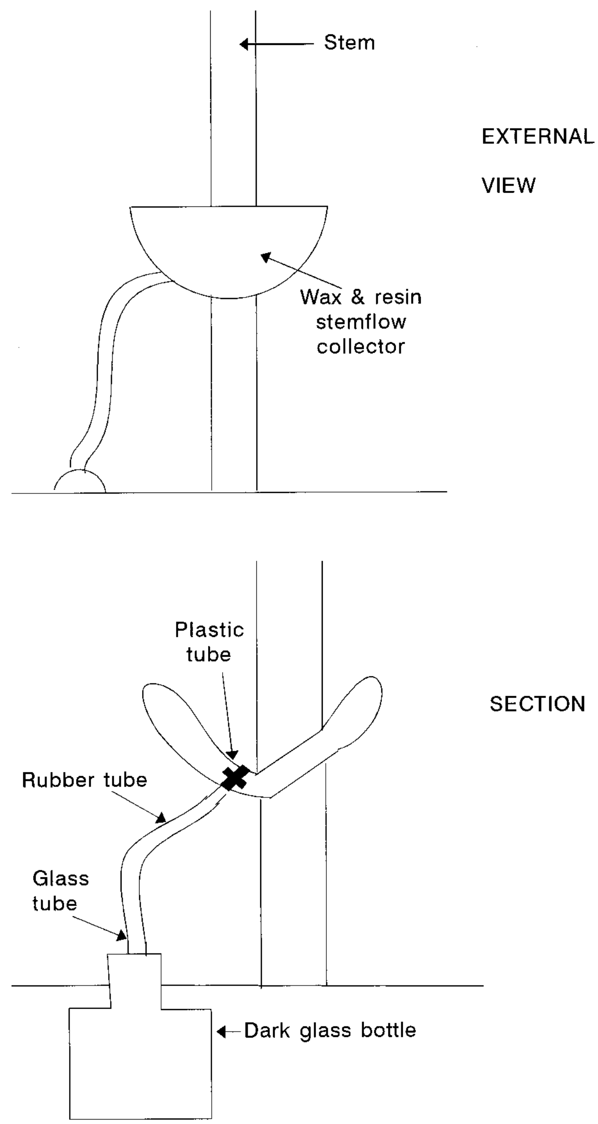

Figure 1. Arrangement of stemflow collectors in the stems. Those collectors were done in the field, avoiding effects on the *Acacia dealbata* and allowing the capture of all stemflow. See text for a complete explanation.

In order to study the effect of percolates through the surface of the ground, two plastic containers were buried 30 cm deep, with care to avoid damage to the soil structure. These containers, 40 cm wide and 50 cm long, were connected to bottles of 4.3 L, buried deeper but with an easy access. Twenty-four hours before any sample was collected, the collectors were washed with distilled water. Once collected, the samples were quickly biotested.

Bioassays

The dilutions obtained from the sampling were biotested using as control the rainfall collected at the adjacent field. The *Lactuca sativa* L. var. Great Lakes was chosen as the sample species for the biotest; it is widely used in the literature and has appropriate characteristics (Rice, 1984). Twenty-five biotests were performed for the throughfall, seventeen for the stemflow and eleven for the percolate, between 3 February 1982 and 2 February 1983, including controls in each case.

Five Petri dishes 12 cm diameter were sowed for treatment, including the control. Fifty *L. sativa* seeds were placed on a piece of Whatman 3MM paper moistened with 7.5 ml of the test solution. All the dishes were kept in the dark with constant humidity and temperature (28ºC, RH 80%) for 48 h, and they were afterward stored in the refrigerator for at least 4 h in order to stop growth. The germinated seeds in each dish were counted and radicle length was measured. pH, conductivity, and concentration of Ca, Mg, K, Na were measured in the natural leachates by atomic absorption spectrometry.

Statistical Analysis

The test results were analyzed in a one-way analysis of variance (Sokhal and Rohlf, 1979). An analysis of multiple regression was performed (Pimentel, 1979) with values from the germination or radicle growth of each leachate tested. The independent variables were quantity of throughfall, stemflow, and percolate collected in each plot, conductivity of the liquid samples, pH, concentration of the ions which were measured, daily precipitation of each of the seven days previous to the collection, highest intensity of the washing (we take the highest quotient from the division of rainfall in a shower per the time that it lasted), time of washing, temperature of the day, approximate percentage of flowers in the plant canopy (from 0 to 100%) and a flower's maturity index (also from 0 to 100%), indicating the approximate percentage of opened flowers.

Results and Discussion

Germination and radicle growth test results performed with the throughfall are shown in Figure 2. There are six statistically significant germination inhibition values (marked with a letter and smaller than 100%) and just one promotion, while the growth of the radicles presents seven significantly stimulating assays and five inhibiting ones. All assays during the bloom time showed inhibition of both parameters. The blossom corresponds directly to bioassays 2 & 3 (bloom peak) and 4 to 8 (flower fall). These dates coincide with the period of germination of many autochthonous herbaceous species common in the undergrowth of native tree species that are excluded in the undergrowth of *A. dealbata* (Reigosa et al., 1984).

The results related to the stemflow biotests are summarized in Figure 3. We can see that the inhibition stage reached is generally greater than that produced by throughfall. Almost all of the germination bioassays show

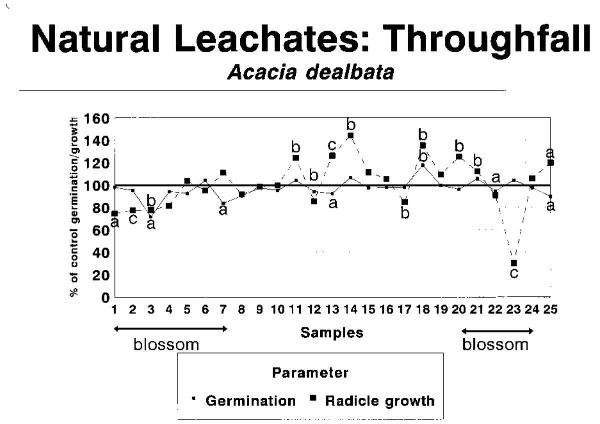

Figure 2. Germination and radicle growth of *Lactuca sativa* biotests using throughfall. Each point represents the mean of the five replications, related to the control. Letters represent statistical differences with control (one control with five replications was performed in each bioassay) according to the oneway ANOVA and LSD test for mean differences. Bonferroni probability levels have been used: a means 5% probability level, b means 1% and c means 1/1000 probability level.

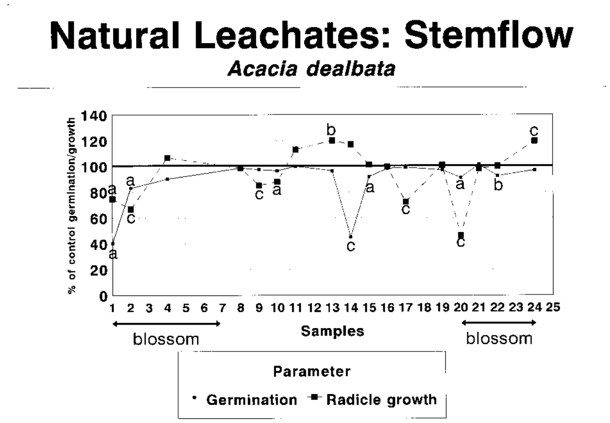

Figure 3. Germination and radicle growth of *Lactuca sativa* biotests using stemflow. Each point represents the mean of the five replications. Letters represent statistical differences with control (one control was performed in each bioassay) according to the oneway ANOVA and LSD test for mean differences. Bonferroni probability levels have been used: a means 5% probability level, b means 1%, and c means 1/1000 probability level.

Table 1. Summary of results in multiple regression analysis (only significant results are shown).

	Multiple r^2	Factors with positive values	Factors with negative values
Radicle growth bioassays as affected by throughfall	0.9514	Rain fallen the same day, the day before and six days before, Ca concentration in the rainfall	Rainfall pH, time of rain, rain fallen four days before, K concentration in the control
Germination bioassays as affected by throughfall	0.8403	Rain fallen three and seven days before, time of rain	Ca rainfall concentration, rain fallen 4 and 6 days before
Radicle growth bioassays as affected by stemflow	0.9347	Rain fallen two days before, . K concentration in the rain	Stemflow quantity, Mg stemflow concentration, Na rain concentration
Germination bioassays as affected by stemflow	0.9974	Rain fallen seven days before, Ca rain concentration	Na rain and stemflow concentration, rain intensity, rain fallen 4 days before
Radicle growth bioassays as affected by percolates	0.9036	Percolate pH, rain intensity	None
Germination bioassays as affected by percolates	0.9931	Percolate conductivity	Rain fallen five days before

inhibition, with six of them being statistically significant. The assays during the blossom period turn out to be especially inhibitory. The effects on the growth of radicles are similar, although a bit less strong. In any case, until June (sample number 13) all cases show inhibition, alternating afterwards between stimulating and inhibitory effects.

The results of the bioassays performed with soil percolates are shown in Figure 4. In germination as well as in radicle growth, these samples are the most inhibitory of all natural washings. The inhibitory results of the pre-blossom period in 1983 appear again. The results of the multiple regression analysis, summarized in Table 1, rule out pH as responsible for the effects produced by natural washings. Conductivity does not appear to be important either. The factors which do appear in a constant way in the different regression equations are those related to the effectiveness of the washing (quantity of rainfall on different previous days, time, and washing intensity). It seems to be proved that small quantities of rainfall can be very effective (Turner and Quarterman, 1975). Squires and Trollope (1979) state that periods without washings allow the accumulation of toxins washable with the first rainfall; Gliessman and Muller (1972) indicate that with consecutive rainfalls of the very same intensity the inhibitory effect of the first rain is the most important. In the later rainfalls the concentration of toxins diminishes. Our results do not support this, however, because it seems that each rainfall washes from the plants effective phytotoxic concentrations.

Phenological data do not form part of the regression equations due to multicolineality, but maturity of the flowers shows a negative correlation to germination and to radicle growth in all tests performed. Flowers seems to play a crucial role in the allelopathic interference of *A. dealbata*, by means of live flower washings and by the flower's soil decomposition.

We want to emphasize the importance of the species producing allelochemicals having a peak of toxicity very early, probably due to the extraordinarily early blossom period of the *Acacia dealbata* in relation to the autochthonous undergrowth species (Rabotnov, 1974; Reigosa et al., 1984). This blossom seems to be related to an increase of phytotoxic capacity and coincides in time with the undergrowth species' germination period.

The experiments carried out allow us to affirm that allelopathic phenomena probably occur in the field.

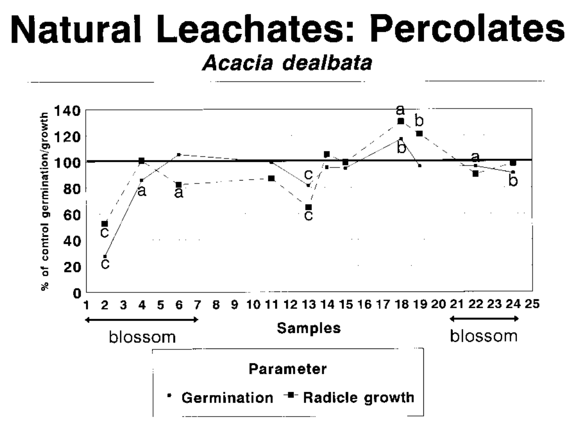

Figure 4. Germination and radicle growth of *Lactuca sativa* biotests using soil percolates. Each point represents the mean of the five replications. Letters represent statistical differences with control (one control was performed in each bioassay) according to the oneway ANOVA and LSD test for mean differences. Bonferroni probability levels have been used: a means 5% probability level, b means 1%, and c means 1/1000 probability level.

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