



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).

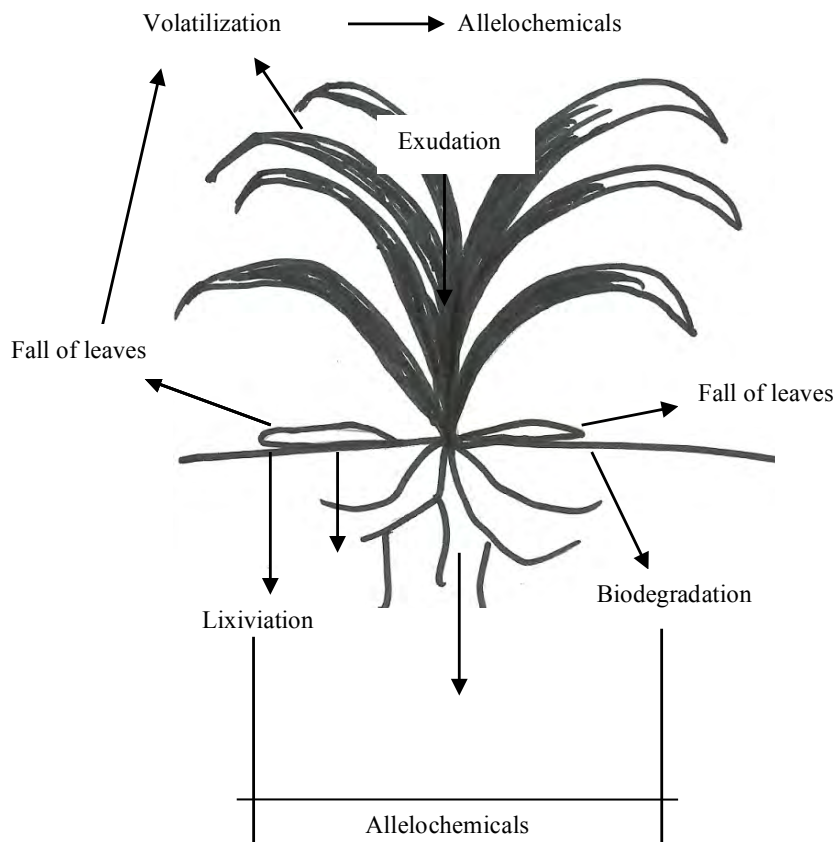


Figure 1.
Ways for the release of allelopathic compounds into the environment.

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

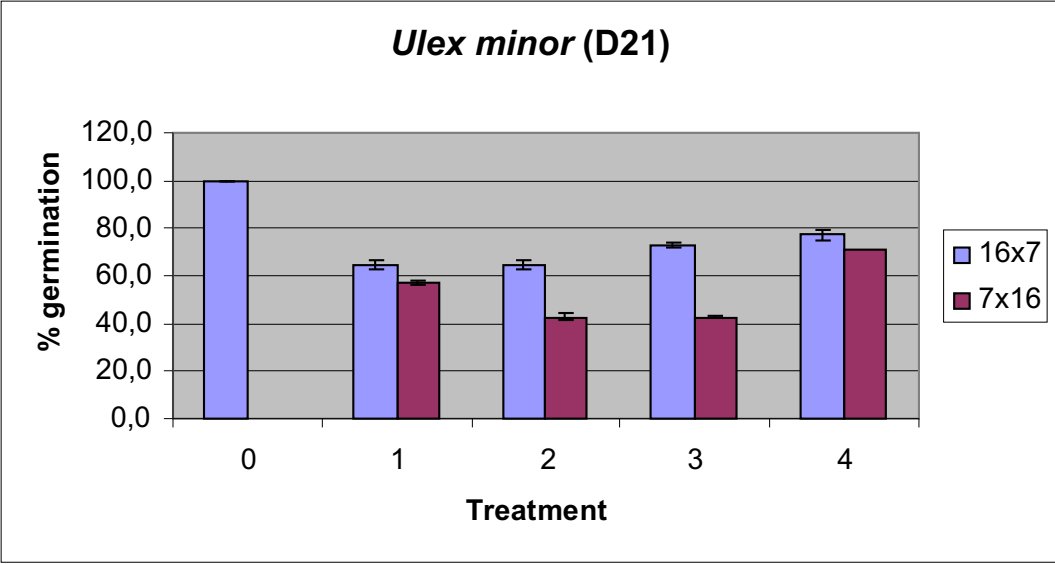


Figure 2.

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

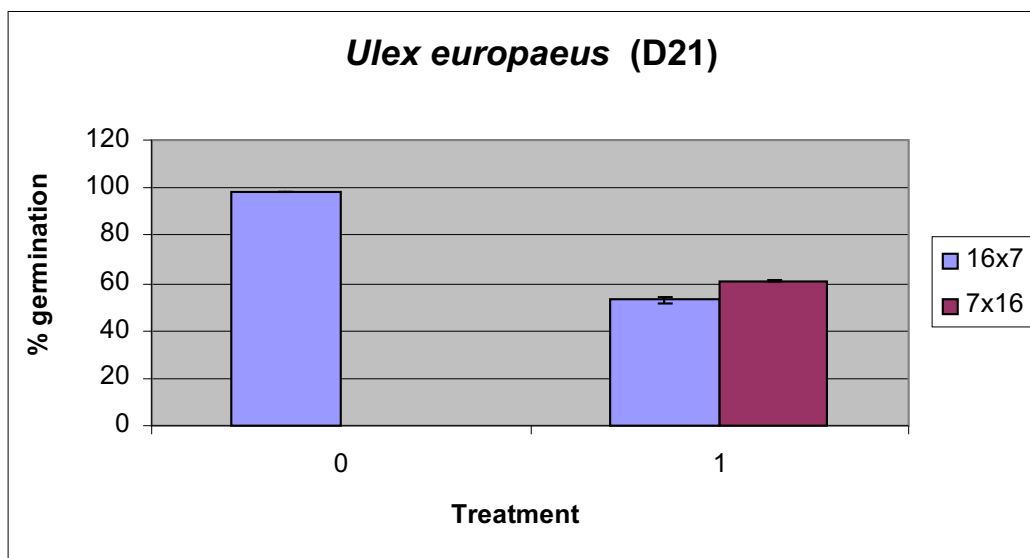


Figure 3.

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

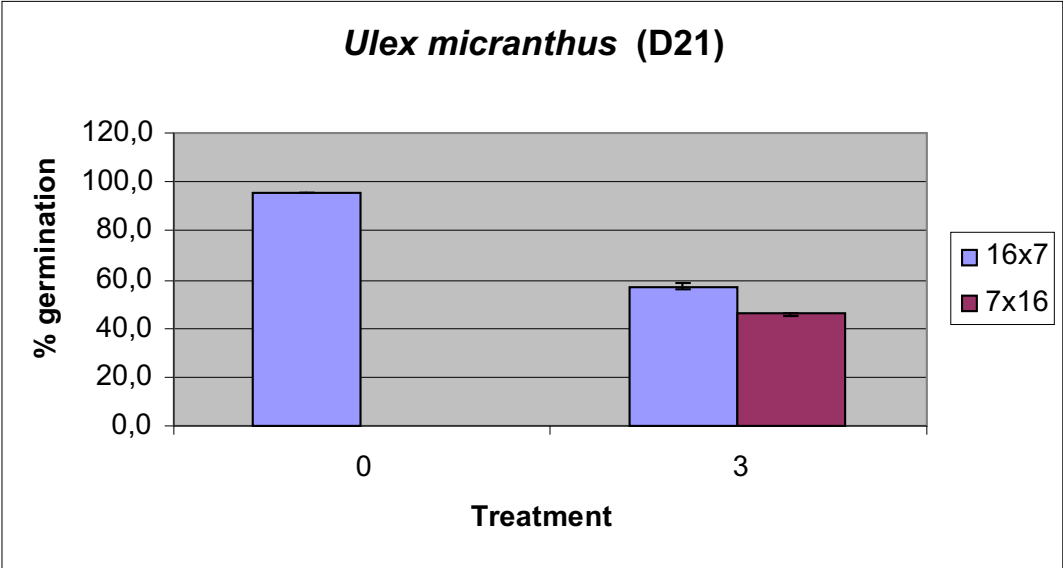


Figure 4. Percentages of germination of *Ulex gallii* on dish after 21 days in isolation (treatment 0) and in concurrent germination with *U. minor* in the two tested proportions. The bars represent the typical deviation. The asterisks indicate significant differences of 5% with regard to the control.

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.

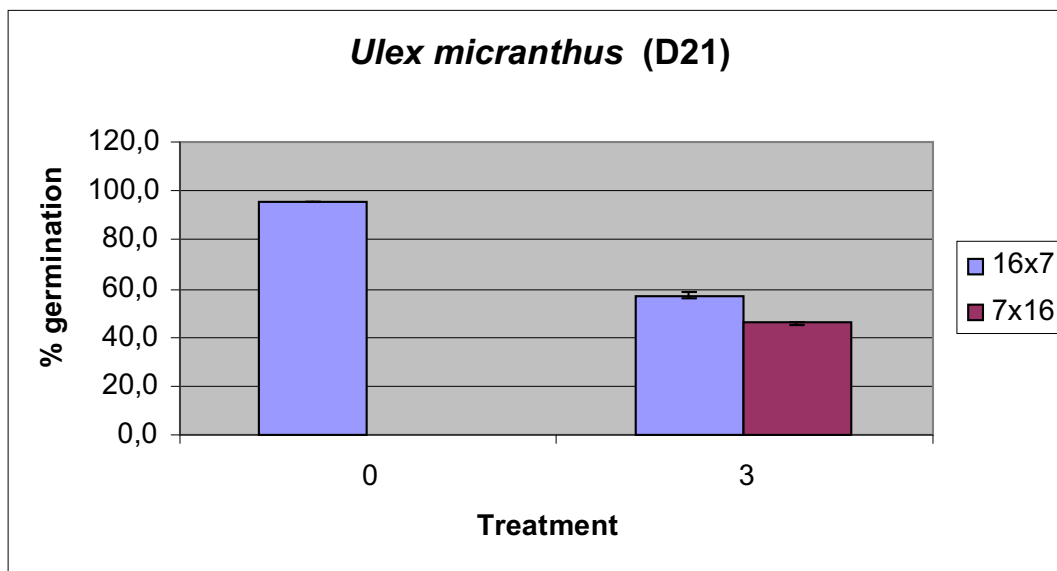


Figure 5.

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.

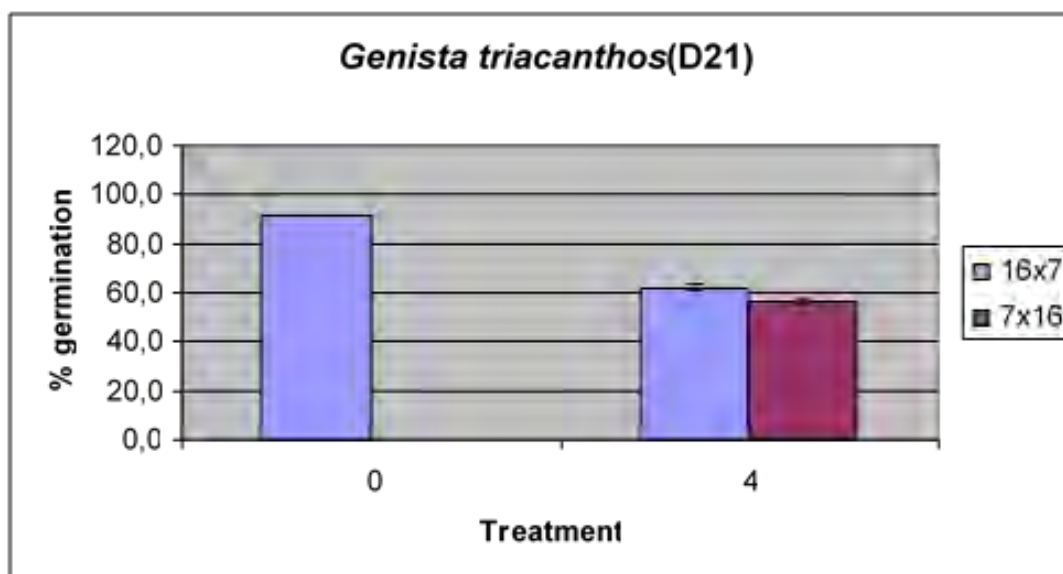


Figure 6.

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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