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

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ABSTRACT

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

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

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

Introduction

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

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

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

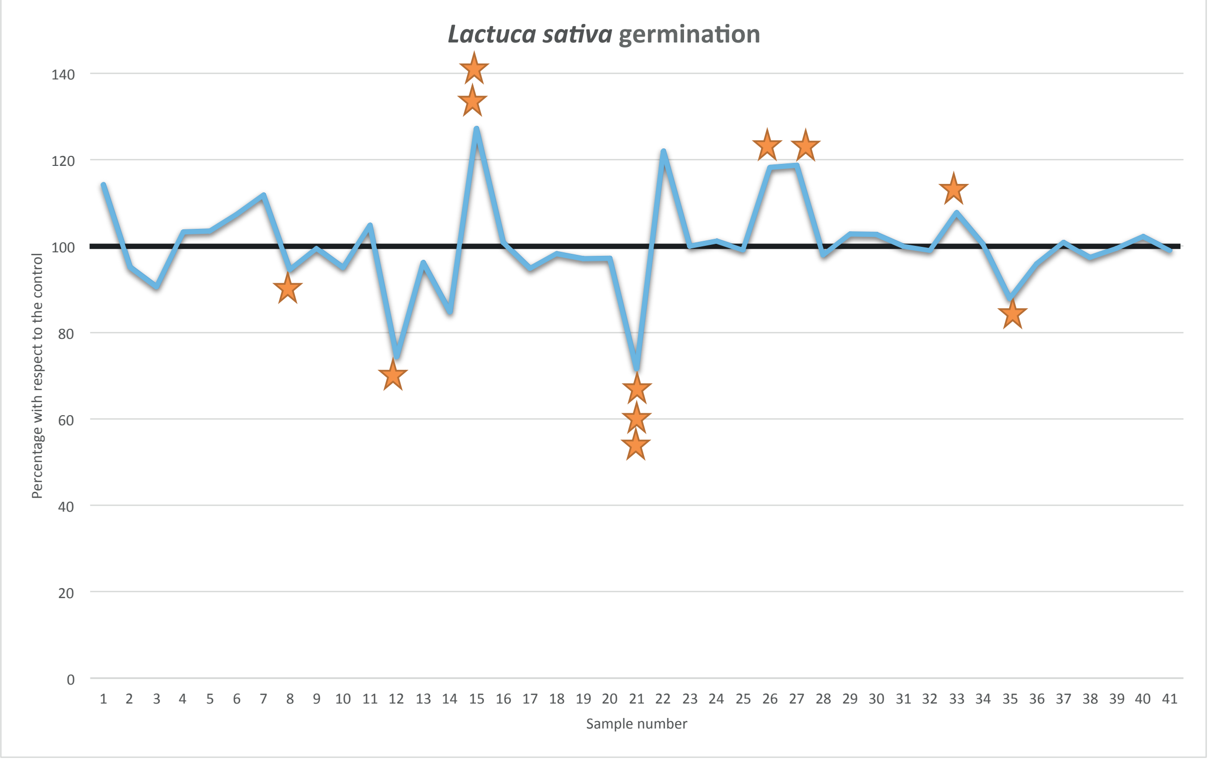

Figure 1.

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

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

The works aimed at clarifying the temporal evolution of phytotoxicity are not very large either (Carballeira & Cuervo, 1980; Casal *et al.*, 1985; Lodhi, 1975, 1976; MacPherson & Muller, 1969; Reigosa *et al.*, 1984, 1991, 2016; Robinson, 1971; Weidenhamer & Romeo, 1989). These diachronic studies are necessary, as the concentration of phytotoxins in the soil is very variable pursuant to the phenological cycle of the producing plant and the environmental factors, this lead to the fact that the measured values are not at all representative (Jalal & Read, 1983). Such variations can even appear in a particular phenological stage and in periods as short as one week (Reigosa *et al.*, 1984). Additionally, there is the possibility of a temporal accumulation of toxins in short but critical periods, by considering the vital cycle stages of compounds release on the part of the plant and the washing periods (Leon, 1976; Quarterman, 1973).

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

Material and methods

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

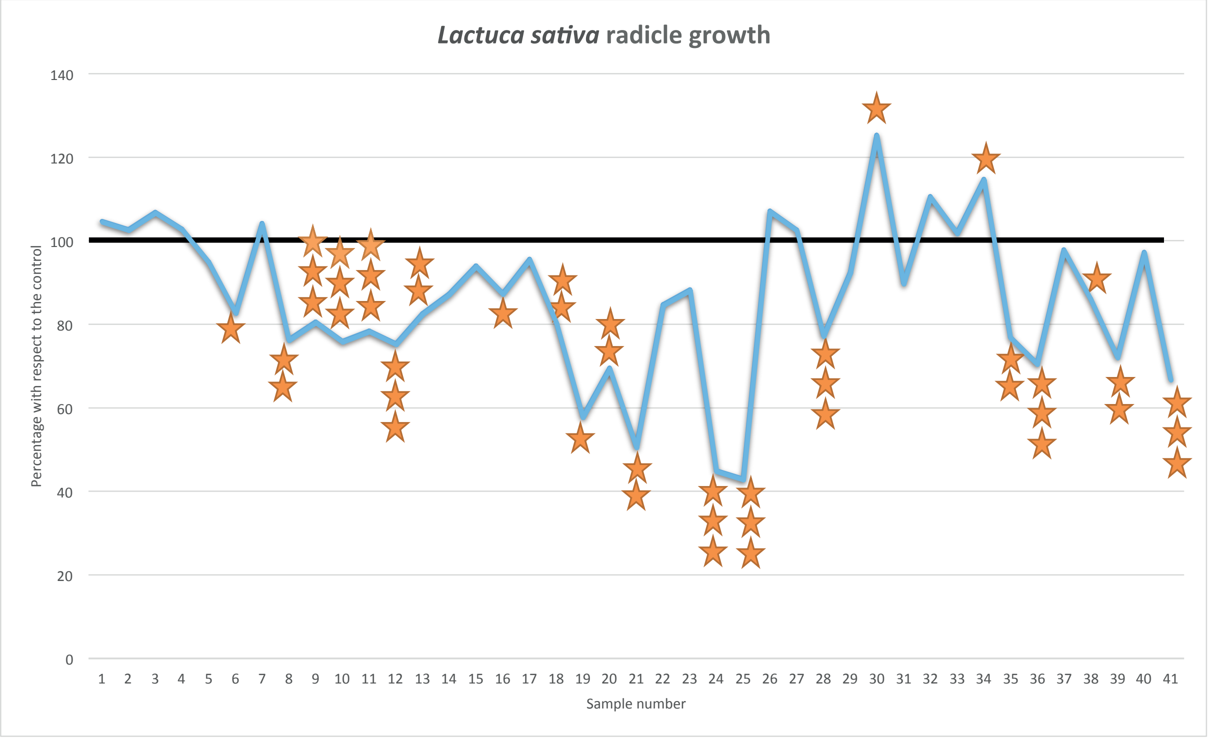

Figure 2.

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

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

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

Results and discussion

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

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

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

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

R multiple	0.3612				
Square R multiple	0.1305				
Square R adjusted	0.0859				
Standard Error of Estimate	17.9005				
Analysis of variance					
	Sum of squares	D.F.	Mean Square	F ratio	
Regression	1875.56780	2	937.7839	2.93	
Residue	12496.72500	39	320.42880		
Variables in the equation:					
Variable	Coefficient	Estándar error of coefficient	st. Reg. Coef	Tolerance	F to remove
Rain 3 days before	0.63199	0.3790	0.249	0.99997	2.78
Temperature	-1. 03498	0.5937	0.260	0.99997	3.04

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

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

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

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

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

References

Afifi, A.A., Azen, S.P. (1979) Statistical analysis. A computer oriented approach. Academic Press.

Ballester, A. (1972) Alelopatía: Interacción química entre especies vegetales. Acta Científica Compostelana, 9 (34): 141145.

Belz, R., van der Laan, M., Reinhardt, C.F., Hurle, K. (2009) Soil degradation of Parthenin-Does it Contradict the Role of Allelopathy in the invasive Weed *Parthenium hysterophorus* L.? Journal of Chemical Ecology, 35(9): 1137-1150.

Carballeira, A., Cuervo, A. (1980) Seasonal variation in allelopathic potential of soils from *Erica australis* L. heathland. Acta Oecologica, Oecologia Plantarum, 1 (15) (4): 345353.

Carballeira, A., Reigosa, M.J. (1999) Effects of natural leachates of *Acacia dealbata* Link in Galicia (NW Spain). Botanical Bulletin of Academia Sinica, 40(1): 87-92.

Casal, J.F., Reigosa, M.J., Carballeira, A. (1985) Potentiel allélopathique de *Acacia dealbata* Link. Rev. Écol. Biol. Sol, 22(1): 112.

Chiapusio, G., Sánchez-Moreiras, A., Reigosa, M.J., Pellissier, F. (1997) Do germination indices adequately reflect allelochemical effects on the germination process? Journal of Chemical Ecology, 23(11): 2445-2453.

Del Moral, R., Muller, C.H. (1969) Fog drip: A mechanism of toxin transport from *Eucalyptus globulus*. Bull. Torrey Bot. Club, 96 (4): 467475.

Gant, R.E., Clebsch, E.E.C., (1975) The allelopathic influences of *Sassafras albidum* in oldfield succession in Tennessee. Ecology, 56: 604615.

Gong, J.T., Li, K.M., Sun, M., Liu, H.J., Zhang, Z.L. (2016) Allelopathy and soil sickness in continuous cropping of *Panax* medicinal plants. Allelopathy Journal, 39(1): 1-17.

González, L., Souto, X.C., Reigosa, M.J. (1995) Allelopathic effects of *Acacia melanoxylon* R.Br. phyllodes during their decomposition. Forest Ecology and Management, 77(1-3): 53-63.

Grant, W.D. (1976) Microbial degradation of condensed tannins. Science, 193: 11371138.

Grove, S., Haubensak, K.A., Parker, I.M. (2012) Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. Plant Ecology, 213(12): 1869-1882.

Guenzi, W.D., Mac Calla, T. (1966) Phytotoxic substances extracted from soil. Soil Sci. Soc. Amer. Proc., 30: 214216.

Hussain, M.I., González, L., Reigosa, M.J. (2011a) Allelopathic potential of *Acacia melanoxylon* on the germination and root growth of native species. Weed Biology and Management, 11(1): 18-28.

Hussain, M.I., González, L., Souto, C., Reigosa, M.J. (2011b) Ecophysiological responses of three native herbs to phytotoxic potential of invasive *Acacia melanoxylon* R.Br. Agroforestry Systems, 83(2): 149-166.

Inderjit, Bhowmik, P.C. (2004) Sorption of benzoic acid onto soil colloids and its implications for allelopathy studies. Biology and Fertility of Soils, 40(5): 345-348.

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. Plant and Soil, 70: 273286.

Jalal, M.A.F., Read, D.J., Haslam, E. (1982) Phenolic composition and its seasonal variation in *Calluna vulgaris*. Phytochem., 22 (6): 13971401.

Jameson, D.A. (1970) Degradation and accumulation of inhibitory substances from *Juniperus osteoperma* (Torrey) Little. Plant and Soil, 33: 213224.

Jose, S. Gillespie, A.R. (1998) Allelopathy in black walnut (*Juglans nigra* L.) alley cropping. I. Spatio-temporal variation in soil juglone in a black walnut-corn (*Zea mays* L.) alley cropping system in the Midwestern USA. Plant and Soil, 203(2): 191-197.

Kaminsky, R. (1981) The microbial origin of the allelopathic potential of *Adenostoma fasciculatum* H & A. Ecol. Monogr., 51 (3): 365382.

Koeppe, D.E., Rohrbaugh, L.H., Rice, E.L., Wender, S.H. (1970a) The effect of Xradiation on the concentration of scopolin and caffeoylquinic acids in tobacco. Radia. Bot., 10: 261265.

Koeppe, D.E., Rohrbaugh, L.H., Rice, E.L., Wender, S.H. (1970b) The effect of age and chilling temperatures on the concentration of scopolin and caffeoylquinic acids in tobacco. Physiol. Plant., 23: 258266.

Koeppe, D.E., Southwick, L.M., Bittell, J.E. (1976) The relationship of tissue chlorogenic acid concentrations and leaching of phenolics from sunflowers grown under varying phosphate nutrient conditions. Can. J. Bot., 54: 593599.

Kung, F. (1971) Decomposition of vanillin by soil microorganisms. Folia Microbiol. (Prague), 16: 4150.

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.

Levitt, J., Lovett, J.V., Garlick, P.R. (1984) *Datura stramonium* allelochemicals: longevity in soil, and ultraestructural effects on root tip cells of *Helianthus anuus* L.. New, 97: 213218.

Lodhi, M.A.K. (1975) Soilplant phytotoxicity and its possible significance in patterning of herbaceous vegetation in a bottomland forest. Am. J. Bot., 62: 618622.

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. Am. J. Bot., 63: 18.

Lorenzo, P., González, L., Reigosa, M.J. (2010) The genus Acacia as invader: the characteristic case of *Acacia dealbata* Link in Europe. Annals of Forest Science, 67(1): 101.

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.

Lorenzo, P., Pazos-Malvido, E., González, L., Reigosa, M.J. (2008) Allelopathic interference of invasive *Acacia dealbata*: Physiological Effects. Allelopathy Journal, 22(2): 453-463.

Lorenzo, P., Pazos-Malvido, E., Reigosa, M.J., González, L. (2010) Differential responses to allelopathic compounds released by the invasive *Acacia dealbata* Link (Mimosaceae) indicate stimulation of its own seed. Australian Journal of Botany, 58(7): 546-553.

Lorenzo, P., Pazos-Malvido, E., Rubido-Bará, M., 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.

Lorenzo, P., Pereira, C.S., Rodríguez-Echevarría, S. (2013). Differential impact on soil microbes of allelopathic compounds released by the invasive *Acacia dealbata* Link. Soil Biology & Biochemistry, 57: 156-163.

MacPherson, J.K., Muller, C.H. (1969) Allelopathic effects of *Adenostoma fasciculatum*, "Chamise", in the California Chaparral. Ecol. Monogr., 39: 177198.

Molina, A., Reigosa, M.J., Carballeira, A. (1991) Release of allelochemical agents from litter, throughfall, and topsoil in plantations of *Eucalyptus globulus* Labill in Spain. Journal of Chemical Ecology, 17(1): 147-160.

Muller, C.H., Chou, C.H. (1972) Phytotoxins: an ecological phase of phytochemistry. In: Phytochemical Ecology, pp. 201216.

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: 621647.

Patrick, Z.A. (1971) Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci., 111: 1318.

Pimentel, A. (1979) Morphometrics. The multivariate analysis of biological data. Kendall / Hunt Publishing Company. Dubuque, Iowa.

Politycka, B. (2005) Soil sickness and allelopathy. Allelopathy Journal, 16(1): 77-84.

Quarterman, E. (1973) Allelopathy in cedar glade plant communities. J. Tenn. Acad. Sci., 48: 147150.

Rabotnov, T.A. (1974) On the allelopathy in the phytocenoses. Izo Acad Nauk SSSR Ser. Biol., 6: 811820.

Reigosa, M.J., Carballeira, A. (2016) Phenological phase influences allelopathic potential of water leachates of *Acacia dealbata* obtained using simulated rain. Journal of Allelochemical Interactions, 2(2): 9-15

Reigosa, M.J., Casal, J.F., Carballeira, A. (1984) Efectos alelopáticos de *Acacia dealbata* Link durante su floración. Studia Oecologica, V: 135150.

Reigosa, M.J., González, L. (2006) Forest ecosystems and allelopathy. In: Allelopathy, a physiological process with ecological implications. Pag. 451-463.

Reigosa, M.J., Sánchez-Moreiras, A., González, L. (1999) Ecophysiological approach in allelopathy. Critical Review in Plant Sciences, 18(5): 577-608.

Reigosa, M.J., Souto, X.C., González, L. (1999) Effect of phenolic compounds on the germination of six weeds species. Plant Growth Regulation, 28(2): 83-88.

Rice, E.L. (1974) Allelopathy. Academic Press.

Rice, E.L. (1979) Allelopathy An update. Bot. Rev., 4 (1): 15109.

Rice, E.L. (1984) Allelopathy. Academic Press.

Robinson, R.K. (1971) Importancia de la toxicidad del suelo en relación con la estabilidad de comunidades de plantas. In: The Scientific Management. Internal Department Translation.

Robinson, R.K. (1972) The production by roots of *Calluna vulgaris* of a factor inhibitory to growth of some micorrhizal fungi. J. Ecol., 60: 219224.

Shindo, H., Kuwatsuka, H. (1977) Behavior of phenolic substances in the decaying process of plants. VI. Changes in quality and quantity of phenolic substances in the decaying process of rice straw in a soil. Soil Sci. Plant Nutr., 23: 319332.

Sokhal, R.R., Rohlf, F.J. (1979) Biometría. Principios y métodos estadísticos de la investigación biológica. Ed. H. Blume.

Souza-Alonso, P., González, L., Cavaleiro, C. (2014) Ambient has become strained, Identification of *Acacia dealbata* Link Volatiles interfering with germination and early growth of Native Species. Journal of Chemical Ecology, 40 (19): 1051-1061.

Stevenson, F.J. (1967) Organic acids in soil. In: Soil Biochemistry (A.B. Mclaren & G. H. Peterson, eds.), 119142. Dekker, New York.

Stowe, L.G. (1979) Allelopathy and its influence on the distribution of plants in an Illinois oldfield. J. Ecol., 67: 10651085.

Tack, B.F., Chapman, P.J., Dagley, S. (1972) Metabolism of gallic and syringic acids by *Pseudomonas putida*. J. Biol. Chem., 247: 64306443.

Teasdale, J.R., Rice, C.F.P., Cai, G., Mangum, R.W. (2012) Expression of allelopathy in the soil environment: soil concentration and activity of benzoxazinoid compounds released by rye cover crop residue. Plant Ecology, 213(12): 1893-1905.

Turner, J.A., Rice, E.L. (1975) Microbial decomposition of ferulic acid in soil. *J. Chem. Ecol*., 1: 4158.

Vaughan, D., Sparling, G.P., Ord, B.B. (1983) Amelioration of the phytotoxicity of phenolic acids by some soil microbes. Soil Biol. Biochem., 15 (5): 613614.

Wang, T.S.C., Yeh, K.L., Cheng, S.Y., Tang, T.K. (1971) Behavior of soil phenolic acids. In: Biochemical interactions among plants, Natl. Acad. Sci., Washington, D.C.

Weidenhamer, J.D. Romeo, J.T. (1989) Allelopathic Properties of *Poligonella myriophylla*. Field Evidence and Bioassays.

J. Chem. Ecol., 15 (7): 19571970.

Whitehead, D.C. (1964) Identification of phydroxybenzoic, vanillic, pcoumaric and ferulic acids in soils. Nature, 202: 417418.

Xiao, H.L., Peng, S.L., Mo, J.M., Chen, Z.Q., Wu, J.R. (2007) Relationships between the allelopathy and nutrient contents in plant and soil. Allelopathy Journal, 19(2): 297-310.

Zhang, Q., Yu, X. (2001) Allelopathy in replant problem in forest soil. Allelopathy Journal, 8(1): 51-64.

Zhang, Q.S. (1993) Potential role of allelopathy in the soil and the decomposing root of Chinese-Fir replant woodland. Plant and Soil, 151(2): 205-210.

Zhang, S.S, Jin, Y.L., Tang, J.J., Chen, X. (2009) The invasive plant *Solidago canadensis* L. suppresses local soil pathogens through allelopathy. Applied Soil Ecology, 41(2): 215-222.

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