Journal of Chemical Ecology, Vol. 20, No. 11, 1994

COMPARATIVE ANALYSIS OF ALLELOPATHIC EFFECTS PRODUCED BY FOUR FORESTRY SPECIES DURING DECOMPOSITION PROCESS IN THEIR SOILS IN GALICIA (NW SPAIN)

X.C. SOUTO,[1] L. GONZALEZ,[2] and M.J. REIGOSA[2,*]

[1]*Departmento de Recursos Naturais e Medio Ambiente*
Area de Producción Vexetal
E.U.I.T. de Industrias Forestais
Universidade de Vigo
Apdo. 874. 36200 Vigo, Spain

[2]*Departamento de Recursos Naturais e Medio Ambiente*
Area de Bioloxía Vexetal
Facultade de Ciencias, Universidade de Vigo
Apdo. 874. 36200 Vigo, Spain

(Received May 12, 1994; accepted July 11, 1994)

Abstract—The development of toxicity produced by vegetable litter of four forest species (*Quercus robur* L., *Pinus radiata* D.Don., *Eucalyptus globulus* Labill, and *Acacia melanoxylon* R.Br.) was studied during the decomposition process in each of the soils where the species were found. The toxicity of the extracts was measured by the effects produced on germination and growth of *Lactuca sativa* L. var. Great Lakes seeds. The phenolic composition of the leaves of the four species was also studied using high-performance liquid chromatographic analysis (HPLC). It was verified that toxicity was clearly reflected in the first stages of leaf decomposition in *E. globulus* and *A. melanoxylon*, due to phytotoxic compounds liberated by their litter. At the end of half a year of decomposition, inhibition due to the vegetable material was not observed, but the soils associated with these two species appeared to be responsible for the toxic effects. On the other hand, the phenolic profiles are quite different among the four species, and greater complexity in the two toxic species (*E. globulus* and *A. melanoxylon*) was observed.

Key Words—Allelopathy, decomposition, litter, *Quercus robur* L., *Pinus radiata* D.Don., *Eucalyptus globulus* Labill, *Acacia melanoxylon* R.Br., phenolics.

*To whom correspondence should be addressed.

INTRODUCTION

Many metabolic products that may be involved in plant–plant chemical interactions are released from plants primarily through leachates from living aboveground parts and later by litter fall. These play a significant role in the plants' distribution.

Forest litter has been recognized as a possible cause for differences in germination and growth of plant species beneath trees of various species (Kuiters, 1989; Souto et al., 1992). Basanta et al., (1989), and Rigueiro and Silva-Pando (1983), who investigated the structure and diversity in the undergrowth of oak woods and other arboreal communities, concluded that there were great differences among them. In Galicia (NW of Spain), *Quercus robur* is an autochthonous species, and it is dominant in climax forests, whereas *Pinus radiata*, *Eucalyptus globulus*, and *Acacia melanoxylon* are foreign species, introduced for commercial purposes. The exotic character of these three species could play an important role in their toxic capacity (Rabotnov, 1974). Leachates also influence the number and behavior of soil microorganisms that affect the soil-forming processes, soil fertility and susceptibility, and immunity of plant species to pests (Gigon and Ryser, 1986).

Phenolic compounds have been considered responsible for allelopathic effects by various authors (Rice, 1984; Inderjit and Dakshini, 1991; Blum et al., 1991). Some authors state that phenolic compounds are present in the majority of the soils associated with forestry ecosystems (Kuiters and Denneman, 1987). Kögel and Zech (1985) found that the phenolic acid composition in the soil humus layer is determined to a high degree by the phenolic substances originating from leaf litter. Therefore, the identification of these types of compounds, particularly phenolics of low molecular weight and flavonoids, was attempted in the leaves of the species studied.

In this work, the allelopathic potential of four forestry species was investigated, and it was attempted to relate this effect to the low vegetative diversity in the undergrowth of some of these species. The study also attempted to see if interactive effects existed between soil leaf litter and microbiotic activity in each soil. Finally, the phenolic profiles of the leaves of the four species were compared before investigating the compounds that impart toxicity in the soils during decomposition of the litter.

METHODS AND MATERIALS

The plots studied were an autochthonous oak wood (*Quercus robur*), an eucalyptus crop (*Eucalyptus globulus*), an acacia crop (*Acacia melanoxylon*), and a pine crop (*Pinus radiata*), which, in the NW of the Iberian Peninsula where the study was carried out, are nonindigenous species.

The stands were situated within the coordinates UTM 535,420–4,748, 792 and 535,970–7,748,702 and between 250 and 290 m above mean sea level. The slope varied between 15% and 17%. The distance between them was 300 m, and they were found in an area where the parent material is granite with mica.

In January 1990 recently fallen leaves were collected and taken to the laboratory, homogenized, and air-dried until the tests were carried out. Dry weight was determined by drying in an oven at 100°C until a constant weight for three aliquots of each sample was obtained. This allowed calculation of the fresh weight equivalent to each dry weight.

Decomposition in Natural Soils. The equivalent of 10 g dry weight in fresh weight was put into nylon net bags with 2-mm-diameter pores (McCauley, 1975; Gloaguen and Touffet, 1980; Woods and Raison, 1982; Kelman and Lang, 1982). In this way, 72 bags were filled with plant material of each especies.

Bags were distributed in each stand (autochthonous oak wood, eucalyptus crop, acacia crop, and pine crop) as follows: six bags of each species were buried in the surface soil (first 15 cm) in three different places chosen at random. In total 288 bags were buried.

The litter bags were removed at 1, 7, 15, 30, 180, and 365 days after their burial. At the end of each period, a bag from each of the three points in the plot was removed at random, taken to the laboratory and homogenized so that the decomposing vegetable material could be put into distilled water for 24 hr in the dark at room temperature, keeping a 1 : 1 ratio of weight to volume (grams dry weight per milliliter).

Two other solutions were prepared from the initial extract by adding distilled water to form concentrations of 1 : 2 and 1 : 10 dilution. Measurements of pH and conductivity were taken and the extracts bioassayed with *Lactuca sativa* var. Great Lakes. Three Petri dishes (9 cm diameter) were prepared for treatment with Whatman 3MM paper sown with 50 seeds of the receptor species in each dish.

The dishes were watered with 4 ml of the corresponding solution and kept in an oven at 28°C and constant humidity. After 60 hr, the dishes were taken to a 4°C cold chamber for at least 4 hr in order to stop the growth of the seedlings, after which the percentage germination and length of the emergent radicles were calculated.

The effects produced on the germination and growth of *L. sativa* by distilled water and by macerated decomposing leaves of *Q. robur* buried in their own wood, (1, 7, 15, 30, 180, and 365 days) were taken as controls. There were no significant differences between them, so the last one was taken as a real control. This arboreal formation was considered as a climax stage in the studied area, including also the associated soil flora. The data represented in this work

are a function of the values obtained with oak in an oak woods and are assumed to be 100%.

The data were analyzed for variation using two-way ANOVA as well as an LSD multiple class test. By means of linear regression and correlation, the effects of pH and conductivity on germination and radicle growth were investigated in order to rule this out as the cause of the inhibitions observed. All the statistical analyses were carried out by the statistical program SPSS/PC+.

Extraction, Purification, and Identification of Phenolic constituents. The phenolic composition of leaves of four species was studied. Ten grams of plant material were powdered and shaken with 300 ml of methanol–water (80:20) for 24 hr at room temperature. The extracts were filtered, and the methanol was evaporated under reduced pressure at 28°C. The aqueous solution was extracted with 30 ml of diethilic ether three times, then evaporated to dryness in a vacuum evaporator and finally redisolved in methanol.

The samples were subjected to HPLC analysis. To identify flavonoids, a Hewlett Packard chromatograph equipped with a UV-Diodo Array detector was used. Identification of the compounds was done by using a reverse-phase Hypersil ODS C-18 (4 × 200) column with a 5-μm particle size. Extracts were analyzed using two mobile phases: A, methanol–phosphoric acid (999:1); and B, water–phosphoric acid (999:1). Linear gradients starting at 20% A and ending at 100% A were used over the first 40 min with an additional 5 min at 100% A. The flow rate of the mobile phase was 1 ml/min, and the eluate was analyzed at 250–400 nm.

To identify phenolics of low molecular weight, a Unicam chromatograph equipped with a UV-Diodo Array detector was used. Analyses were made by using a reverse-phase Waters Nova-Pak C-18 (3.9 × 300) column with a 4-μm particle size. Extracts were analyzed using two mobile phases: A, water–acetic acid (98:2); and B, water–methanol–acetic acid (68:30:2). Linear gradients starting at 100% A and ending at 20% A were used over the first 59 min, with an additional 6 min at 20% A. The flow rate of the mobile phase was 0.8 ml/min, and the eluate was analyzed at 210–400 nm.

Identification in both cases were based on retention times and UV spectra of authentic standards.

RESULTS AND DISCUSSION

Effects of Soil Vegetable Material. During the first 30 days of decomposition, the leaves from oak did not show any toxicity in any of the woods where they were buried (Figure 1). On the other hand, the eucalyptus macerates showed strong inhibitory effects both on radicle growth and germination of the seeds,

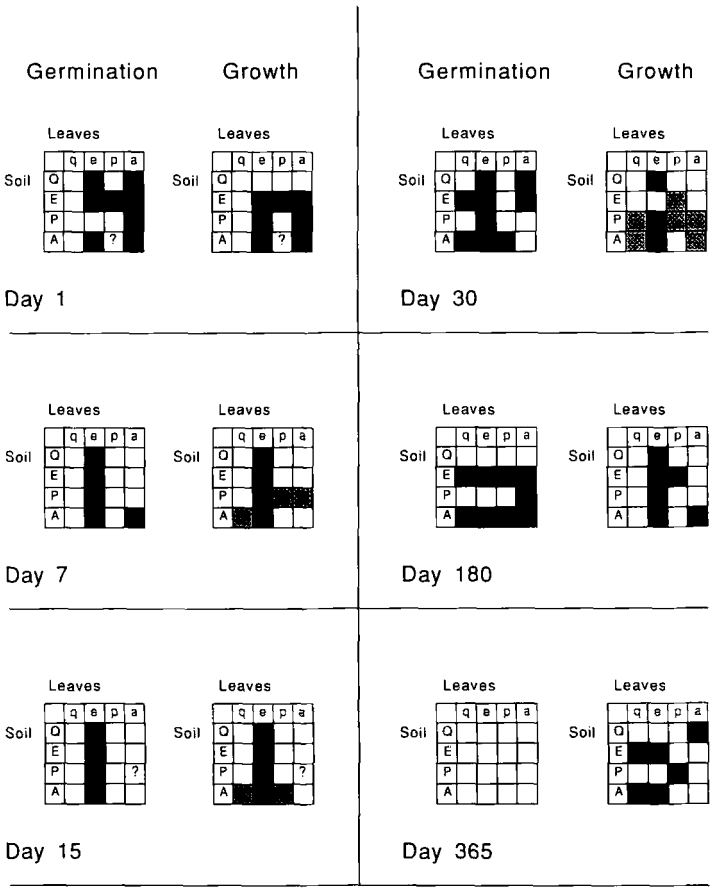

FIG. 1. *Concentration 1.* Evolution of phytotoxicity produced by leaves of four species in decomposition in four different soils, measured as inhibition of germination and growth of the radicles of *Lactuca sativa* seeds. On the horizontal axis, the kind of vegetable matter is represented; on vertical axis the soils where the residues are decomposing are shown. The black squares indicate inhibition with respect to the control (residues of *Quercus robur* decomposing in its own wood) and the grey squares indicate stimulatory effects with respect to the same control, with significant differences at the level of 5% in both cases. **q, e, p,** and **a** indicate leaves of *Quercus robur*, *Eucalyptus globulus*, *Pinus radiata*, and *Acacia melanoxylon*, respectively. **Q, E, P,** and **A** indicate soils where the decomposition process is that of *Quercus robur* wood and *Eucalyptus globulus*, *Pinus radiata*, and *Acacia melanoxylon* crops respectively.

which was independent of the soil where they were buried. This effect continued until 180 days in the case of radicle growth inhibition. Leaf litter from acacia induced strong inhibition (more than 80%) on growth and germination at the beginning of its decomposition.

After 30 days, strong inhibition due specifically to leaf litter was not found but was seen to be more a function of soil type where the decomposition takes place. Thus, even materials from oak and pine, which apparently do not produce toxic effects on their own, are capable of inhibiting germination when they decompose in eucalyptus stands (the case for oak) or in acacia stands (the case for both oak and pine).

At 180 days, the tendency observed 30 days after the start of decomposition was confirmed; that is, all the leaves from all the species show inhibition of germination when they decompose in either eucalyptus or acacia.

At 365 days after the start of decomposition, the inhibitory effects on germination disappeared; however, the toxicity on growth still remained. This seems to indicate that toxicity on germination precedes the inhibition on radicle growth, which is a similar result to that found by Reigosa et al. (1984). Ecologically, it would be more effective for toxic species to show initial inhibition on germination and later on emergent plant growth of plants that have escaped the first control inhibiting their development.

We thus see that eucalyptus and acacia are potentially toxic species. Although the acacia only shows release of toxins at the start of decomposition, it adds vegetable matter to the soil throughout the year. Thus, the toxic effect could be continuous and very important during the germination period of undergrowth species.

Once the starting vegetable material stops being the main cause of toxicity, the soils that induce toxicity are those associated with eucalyptus and acacia stands.

Interactive Effects. At the beginning of the decomposition process and 30 days after the start of the experiment, the interaction between the starting material and different microbiological activity is statistically significant (Table 1). We should also take note of the interaction that can be seen at 365 days only on radicle growth. These analyses indicate that the process of liberation of allelopathic compounds during decomposition does not just depend on the decomposing material and the microbiological activity of the soil, but also on the combination of both effects.

Effects of Concentrations 2 and 3. The results for concentration 2 (Figure 2 and Table 2) maintain the same pattern of variation as the more concentrated treatment, although the inhibitory effects are reduced slightly with respect to concentration 1. An obvious toxicity at the beginning of decomposition of acacia is again seen, which is the same as for the first 30 days in eucalyptus. In the same way, at 180 days the toxicity is basically due to the effect of the soil,

TABLE 1. TWO-WAY ANOVA FOR INHIBITORY EFFECTS OF MACERATED LEAVES DECOMPOSING IN FOUR DIFFERENT SOILS, CONCENTRATION 1 : 1[a]

	Germination			Growth		
Day	Leaves	Soil	Interaction	Leaves	Soil	Interaction
1	***	***	***	***	**	**
7	***	NS	*	***	NS	*
15	***	NS	NS	***	NS	**
30	***	***	***	***	***	***
180	*	***	NS	***	***	NS
365	*	NS	NS	NS	**	***

[a] $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, no significant differences.

where decomposition continues but toxic effects, after a year, are practically nonexistent.

At concentration 3 (Figure 3 and Table 3) a clear predominance of neutral effects is seen. However, despite the strong dilution of the macerate, there is an evident initial difference due to the starting material, where acacia and eucalyptus are clearly toxic. During the decomposition process, this effect is diluted, showing toxicity caused by the soil where the decomposition takes place at the end. As in the more concentrated dilutions toxicity is seen in solutions associated with the eucalyptus and acacia stands.

Phenolic Composition. The phenolic contents in the samples analyzed are shown in semiquantitative mode in Table 4. Large differences between species were observed. Thus, more complex profiles are seen in samples of *E. globulus* and *A. melanoxylon* than in *P. radiata* and *Q. robur*. In the extracts of eucalyptus, gallic, vanillic, ferulic, 3,4,5-trimethoxybenzoic, and ellagic acids; 4-hydroxybenzaldehyde; 4-hydroxyphenethyl alcohol; 4-hydroxy-3-methoxybenzyl alcohol; quercitrin; and quercetin were identified. Due to UV spectra, ellagitannins, flavonols and some flavanones also were observed, although it was not possible to determine their structures. Vanillic and ferulic acids such as vanillin, 4-hydroxy-3-methoxybenzyl alcohol, quercetin-3-glycoside (possibly rutin), quercitrin, luteolin, and apigenin were identified in acacia. Flavonols, flavones, and flavanones were also detected by their UV spectra. Vanillic, *p*-coumaric, ferulic, and ellagic acids; 4-hydroxy-3-methoxybenzyl alcohol; vanillin, quercitrin, and toxifolin were also identified in pine. Flavone was furthermore detected in its spectrum. In oak extracts, 3,4-dihydroxybenzoic, vanillic, and ellagic acids were detected as were 4-hydroxybenzaldehyde, quercetin, and kaempferol.

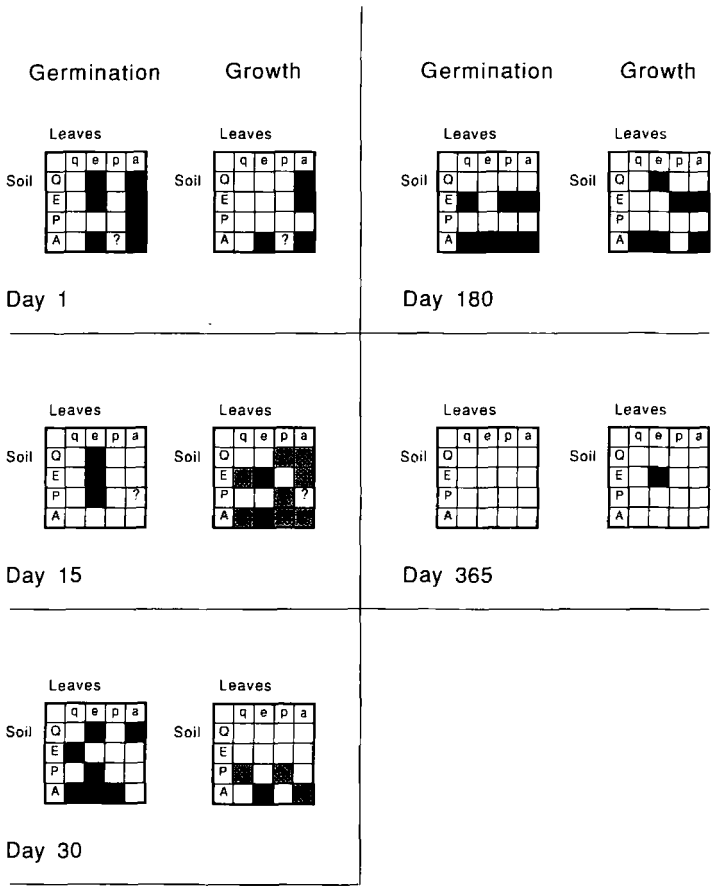

FIG. 2. *Concentration 2.* Evolution of phytotoxicity produced by leaves of four species in decomposition in four different soils, measured as inhibition on the germination and growth of the radicles of *Lactuca sativa* seeds. On horizontal axis, the kind of vegetable matter is represented; on vertical axis, the soils where the residues are decomposing are shown. The black squares indicate inhibition with respect to the control (residues of *Quercus robur* decomposing in its own wood) and the grey squares indicate stimulatory effects with respect to the same control, with significant differences at the level of 5% in both cases. Letter symbols are as in Figure 1.

TABLE 2. TWO-WAY ANOVA FOR INHIBITORY EFFECTS OF MACERATED LEAVES DECOMPOSING IN FOUR DIFFERENT SOILS, CONCENTRATION 1:2[a]

	Germination			Growth		
Day	Leaves	Soil	Interaction	Leaves	Soil	Interaction
1	***	***	***	***	*	**
15	***	NS	NS	***	*	***
30	***	***	***	***	**	**
180	NS	***	NS	NS	**	**
365	NS	NS	NS	NS	*	*

[a] $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, no significant differences.

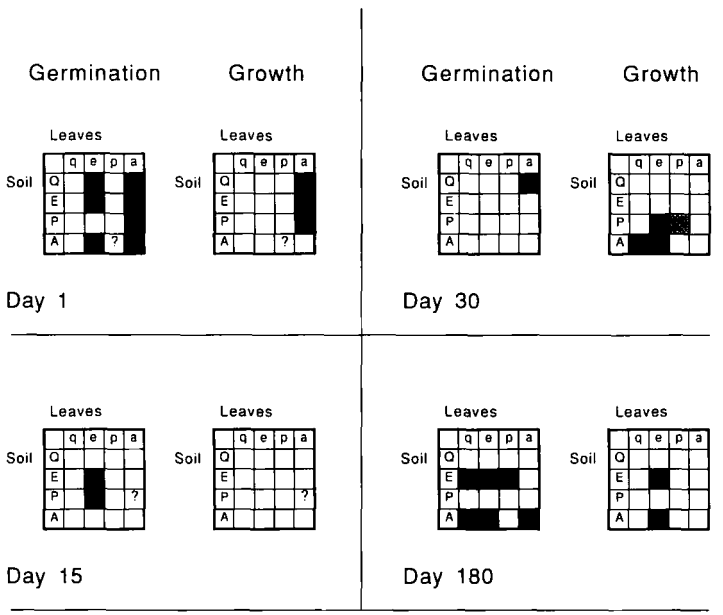

FIG. 3. *Concentration 3.* Evolution of phytotoxicity produced by leaves of four species in decomposition in four different soils, measured as inhibition on the germination and growth of the radicles of *Lactuca sativa* seeds. On horizontal axis, the kind of vegetable matter is represented; on vertical axis, the soils where the residues are decomposing are shown. The black squares indicate inhibition with respect to the control (residues of *Quercus robur* decomposing in its own wood) and the grey squares indicate stimulatory effects with respect to the same control, with significant differences at the level of 5% in both cases. Letter symbols are as in Figure 1.

TABLE 3. TWO-WAY ANOVA FOR INHIBITORY EFFECTS OF MACERATED LEAVES DECOMPOSING IN FOUR DIFFERENT SOILS, CONCENTRATION 1 : 10[a]

	Germination			Growth		
Day	Leaves	Soil	Interaction	Leaves	Soil	Interaction
1	***	NS	**	***	NS	NS
15	NS	NS	NS	NS	NS	NS
30	***	***	***	NS	NS	***
180	NS	***	NS	NS	***	NS

[a] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS, no significant differences.

TABLE 4. PHENOLIC COMPOSITION OF LEAVES OF FOUR FOREST SPECIES: *Quercus robur*, *Eucalyptus globulus*, *Pinus radiata*, and *Acacia melanoxylon*[a]

	Compound	Oak	Eucalyptus	Pine	Acacia
1	Gallic acid	–	+++++	–	–
2	3,4,-Dihydroxybenzoic acid	+	–	–	–
3	Vanillic acid	+	+	+	++
4	4-hydroxybenzaldehyde	+	+	–	–
5	4-hydroxyphenethyl alcohol	–	++	–	–
6	4-hydroxy-3-methoxybenzyl alcohol	–	+	++++	++++++
7	p-coumaric acid	–	–	+	–
8	Vanillin	–	–	+	+
9	Taxifolin	–	–	++	–
10	Ferulic acid	–	+	+	+
11	3,4,5,-Trimethoxybenzoic acid	–	++	–	–
12	Quercetin 3-Glycoside	–	+	+	+++
13	Ellagic acid	++++	+++++	++	–
14	Quercitrin	–	+++	++	++
15	Ellagitannins	++	++++	–	–
16	Quercetin	+++	+++	–	–
17	Kaempferol	+++	–	–	–
18	Luteolin	–	–	–	+++++
19	Apigenin	–	–	–	+++++

[a] Semiquantitative values based on the relative intensity of the chromatographic peaks.

Acknowledgments—We thank the CONSELLERÍA DE EDUCACIÓN E ORDENACIÓN UNIVERSITARIA-X4NTA DE GALICIA for financial support of this work (cód. XUGA 30103B92).

REFERENCES

BASANTA M., DIAZ, E., CASAL, L., and MOREY, M. 1989. Diversity measurements in shrubland communities of Galicia (NW Spain). 82:105–112.

BLUM, U., WENTWORTH, T.R., KLEIN, K., WORSHAM, A.D., KING, L.D., GERIG, T.M., and LYU, S.W. 1991. Phenolic acid content of soils from wheat-no till, wheat-conventional till, and fallow-conventional till soybean cropping systems. *J. Chem. Ecol.* 17:1045–1068.

GIGON, A. and RYSER, P. 1986. Positive interactions between plant species. I. Definition and examples from grassland ecosystems. *Veroeff. Geobot. Inst. Eidg. Tech. Hochsch. Stift. Rubel Zurich* 87:372–387.

GLOAGUEN, J.C., and TOUFFET, J. 1980. Vitesse de décomposition et évolution minérale des litières sous climat atlantique. I. Le hêtre et quelques conifères. *Acta Oecol. Oecol. Plant.* 15:3–26.

INDERJIT, and DAKSHINI, K.M.M. 1991. Hesperetin 7-rutinoside (hesperidin) and toxifolin 3-arabinoside as germination and growth inhibitors in soils associated with the weed, *Pluchea lanceolata* (DC) C.B. Clarke (Asteraceae). *J. Chem. Ecol.* 17:1585–1591.

KELMAN, R., and LANG, G.E. 1982. A critique of the analytical methods used in examining decomposition data obtained from litter bags. *Ecology* 63(6):1636–1642.

KÖGEL, I., and ZECH, W. 1985. The phenolic acid content of cashew leaves (*Anacardium occidentale* L.) and of the associated humus layer. Senegal. *Geoderma* 35:119–125.

KUITERS, A.T. 1989. Effects of phenolic acids on germination and early growth of herbaceous woodland plants. *J. Chem. Ecol.* 15:467–479.

KUITERS, A.T., and DENNEMAN, C.A.J. 1987. Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. *Soil Biol. Biochem.* 19:765–769.

MCCAULEY, 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.

RABOTNOV, T.A. 1974. On the allelopathy in the phytocenoses. *Izv. Akad. Nauk SSSR Ser. Biol.* 6:811–820.

REIGOSA, M.J., CASAL, J.F. and CARBALLEIRA, A. 1984. Efectos alelopáticos de *Acacia dealbata* Link durante su floración. *Stud. Oecol.* 5:135–150.

RICE, E.L. 1984. Allelopathy, 2nd Edition. Academic Press, Orlando, Florida.

RIGUEIRO, A., and SILVA-PANDO, F.J. 1983. Algunas consideraciones sobre los efectos del *Eucalyptus globulus* Labill sobre el medio natural gallego. *Cuad. Area C. Agrarias* 4:426–444.

SOUTO, X.C., GONZALEZ, L., PEDROL, M.N., and REIGOSA, M.J. 1992. Allelochemical effects produced by *Eucalyptus globulus* Labill during the decomposition process in four natural soils in Galicia (NW Spain). A comparative analysis. *Comp. Physiol. Ecol.* 18(3):114–123.

WOODS, P.V., and RAISON, R.J. 1982. An appraisal of techniques for the study of litter decomposition in eucalypt forests. *Aust. J. Ecol.* 7:215–225.