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# The potential effects of allelopathic mechanisms on plant species diversity and distribution determined by the wheat rootlet growth inhibition bioassay in South American plants

## Efectos potenciales de los mecanismos alelopáticos sobre la distribución de la diversidad vegetal, determinados mediante el bioensayo de la inhibición en el crecimento de las raíces del trigo en plantas sudamericanas

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### ABSTRACT

Phytotoxicity of aqueous extracts from 60 plant species from tropical and temperate communities was tested by wheat rootlet growth inhibition assay. Differences in the inhibition and stimulation of the growth of the wheat rootlets were observed among both communities. 26.7 % of the total sample belonging to the tropical community showed considerable allelopathic activity in this bioassay, while 23.3 % of the total sample stimulated rootlet growth. On the other hand, 71.9 % of the temperate plants studied presented this activity, while none of the species showed stimulating effects. The possible influence of allelopathic effects on the coexistence of a particular mixture of plant species in a determined area is discussed.

**Key words:** allelopathy, density dependence, wheat rootlet growth inhibition, plant diversity.

### RESUMEN

Se estudió la actividad fitotóxica de extractos acuosos de 60 especies vegetales pertenecientes a comunidades tropicales y templadas utilizando el ensayo de la inhibición del crecimiento de las raíces de trigo. Se observaron diferencias en la inhibición y estimulación del crecimiento de las raíces de trigo entre ambas comunidades. 26,7% de los extractos de plantas colectadas en una comunidad neotropical mostraron considerable actividad alelopática utilizando este bioensayo, mientras que un 23,3% estimuló el crecimiento radicular. Por otro lado, un 71,9% de las plantas de zonas templadas mostró una actividad alelopática considerable, mientras que en ningún caso se observó estimulación en el crecimiento de las raíces de trigo. Se discute la posible influencia de los efectos alelopáticos en la coexistencia de diferentes especies vegetales en un área determinada.

**Palabras clave:** alelopatía, denso dependencia, diversidad vegetal, inhibición de la elongación de las raíces de los granos de trigo.

## INTRODUCCION

Many plant-plant, plant-animal and plant-pathogen interactions have been described as regulatory functions in the enviroment (Sondheimer & Simeone1970, Whittaker & Feeny 1971, Swain 1977).

Allelopathy, classically defined as the inhibition of the growth or germination of one plant by another through the release into the environment of selectively toxic metabolic by-products, is one of the less studied interactions that may occur among plant species growing together, when it comes to account for the maintenance of high plant diversity.

A decline in the species richness of terrestial plants from the tropics to the polar

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regions has long been recognized (Woodward 1987). Indeed, wet lowland tropical forests characteristically have many plant species and low density of adults of each species compared with temperate-zone forests in habitats of similar areal extent, topographic diversity, and edaphic complexity (Black et al. 1950, Poore 1968, Ashton 1969). However, the underlying reasons for this pattern are still not fully understood. In order to explain high diversity in tropical rain forests, Janzen (1970) and Connell (1971) pointed out that seedlings of tropical woody plants found close to conspecific adults often suffer higher mortality than seedlings found further away. This phenomenon can lead to density dependence in population growth, because as a population increases, more of the forest will be in the zone of high mortality close to adults. Therefore, seedling survival will decrease as the population increases, and this can regulate population size (Condit, 1995).

Several other equilibrium hypotheses have been proposed to account for the maintenance of high diversity (Grubb 1977, Ricklefs 1977, Connel 1979, Chesson and Warner 1981, Tilman 1982, Orians 1983). Each of these models postulates the existence of equilibrating forces that maintain a particular species mixture (Condit et al. 1992). However, although these hypotheses have theoretical merit –each can account for the coexistence of a large number of species– experimental data are not presently available to determine which are important in any particular forest (Condit et al. 1992).

In order to study the potential plant-plant interactions due to allelopathic activity of South-American plant extracts and its influence in the coexistence of different species in a determined area, we used a test inspired by the method of Ceriotti (1965), *i.e.*, the inhibition of the growth of the rootlets of wheat (Boily & Harelimana 1979). This method has proved to be effective in order to determine allelopathic activity of plant extracts, since it provides a direct measurement of the available allelochemicals in the extract (Hance & McKone 1976). A series of 60 plant species were screened. 30 of these plants were collected in the Amazonian rainforest in Madre de Dios, Perú, while the remaining 30 were obtained from the central and southern temperate provinces of Argentina.

## MATERIALS AND METHODS

### *Study site and plant materials*

Plants were collected in the area of the Tambopata river, south-west Amazon basin, aproximately 13°S and 69°30'W, in Madre de Dios, Perú, and in central and southern Argentina, below the latitude of 30°S, in the provinces of Corrientes, Entre Ríos, Santa Fe, Buenos Aires, Chubut and Santa Cruz. Criteria for selection of the plant species was random. In the case of plants representing the tropical region, plants were picked along a trail, while plants of temperate regions were collected during different collection trips in Argentina. Only aerial parts of the plants were selected, and the material was dried under controled conditions (40°C, 10 days) and ground in a blender. Plant material was stored at room temperature for a month before use.

### *Preparation of extracts*

Aqueous extracts were prepared utilizing 10 gr dry weight of plant material to 100 ml of boiling tap water (concentration 100 mg/ml). After 20 min the mixture was filtered under sterile conditions, and pH was controled at a neutral range (6-8).

### *Biological activity*

Grains of wheat were germinated in tap water during 48 h in the dark. Inmediately afterwards, 10 grains were placed on a filterpaper (Whatman no. 1, diameter 9 cm) in a Petri dish (diameter 10 cm) containing 10 ml of the plant infusion (concentration 100 mg/ml). The Petri dishes were then kept in the dark at room temperature and the growth of the rootlets was evaluated after 5 days. Duplicates were performed for all concentrations and controls, and two independent experiments were run. The longest rootlet of each seed was measured and the inhibition calculated as a percentage relative to the length of the rootlets in the control plants growing in tap water. The inhibition of the growth of the rootlets is espressed as percentage compared with the growth of the rootlets of non-treated grains under the same conditions (expressed as the mean of 10 grains). Colchicine ("Sigma", St. Luis, U.S.A) was used as a positive control, at a concentration of 250 ug/ml.

*Data analysis*

The average rootlet length for each concentration was used in growth inhibition calculations. Data outside the +/- 2 SD range were rejected according to González et al. (1993). Plant extracts were considered as having an allelopatic effect when they caused a 90 % or more reduction in the growth of the rootlets, according to the method of Ceriotti (1965). On the other hand, stimulation of growth, was considered in those cases where the relative percentage inhibition was negative. Comparison of two proportions (unpaired case) (Armitage 1973) was used to determine the differences between allelopathic effects among the two communities in study. Since sample sizes were equal to 30, data treatment assumed a normal distribution (Armitage 1973).

## RESULTS

In order to study plant-plant interactions due to allelopathic effects in two communities, 60 plants were screened with the wheat rootlet growth inhibition method (Boily & Harelimana, 1979). Aqueous extracts of the plant material were chosen for this study because of their advantage of more closely simulating field posibilities, such as the activity of leaf and stem leachates. Table 1 shows the results in a sample of 30 plants collected in a neotropical plant community. Eight species of plants (26.7 % of the total sample) showed considerable allelopathic activity in the growth of the wheat rootlets, while 7 plant species (23.3 % of the total sample) stimulated rootlet growth. Table 2 shows the results for a sample of 30 plant species obtained in temperate regions, specifically the central and southern provinces of Argentina. In this sample 22 plant species (71.9 %) presented considerable allelopathic activity, while none of the species showed stimula-

TABLE 1

Inhibition of the growth of the rootlets of wheat by tropical plant extracts at 10% concentration.

Inhibición en el crecimiento de las raíces de los granos de trigo ejercida por extractos de plantas tropicales, a una concentración del 10%.

| Plant: scientific nameand family | Percent inhibition |
|---|---|
| *Abuta grandifolia (Mart.) Sandwith* (Menispermaceae) | -33 %[a] |
| *Aegiphila peruviana Turcz.* (Verbenaceae) | -78 % |
| *Aristolochia triangularis Cham. et Schlecht.* (Aristolocheaceae) | 73 % |
| *Aspidosperma excelsum Benth.* (Apocynaceae) | -50 % |
| *Banisteriopsis caapi (Spruce) Morton* (Malpighiaceae) | 94 % |
| *Clavariadelphus.sp* (Clavariaceae) | 35 % |
| *Campyloneuron phillitidis L.* (Polypodaceae) | 92 % |
| *Cestrum hediondum Dun.* (Solanaceae) | -39 % |
| *Chenopodium ambrosoides L.* (Chenopodiaceae) | 100 % |
| *Cyperus articulatus L.* (Cyperaceae) | 98 % |
| *Dracontium sp* (Araceae) | 80 % |
| *Erythrina ulei Harms* (Leguminoseae) | 14 % |
| *Gallesia integrifolia (Spreng.) Harms.* (Phytolaccaceae) | 68 % |
| *Gentianella alborosea (Gilg) Fabris* (Gentianaceae) | 100 % |
| *Gnaphalium spicatum Lamb.* (Compositae) | 46 % |
| *Heliotropium indicum L.* (Boraginaceae) | -28 % |
| *Jacaranda mimosifolia L.* (Bignonaceae) | 58 % |
| *Jatropha macrantha Muell. Arg.* (Euphorbiaceae) | 100 % |
| *Ouratea sp* (Ochnaceae) | 100 % |
| *Petiveria alliacea L.* (Phytolaccaceae) | 69 % |
| *Phyllanthus niruri L.* (Euphorbiaceae) | 5 % |
| *Piper angustifolium R. et P.* (Piperaceae) | 100 % |
| *Piper callosum R. et P.* (Piperaceae) | -55 % |
| *Pothomorphe peltata (L) Miq.* (Piperaceae) | 50 % |
| *Pseudocalymma alliaceum (Lam.) Sandw.* (Bignonaceae) | -48 % |
| *Triplaris americana L.* (Polygonaceae) | 16 % |
| *Uncaria tomentosa (Willd.) DC.* (Rubiaceae) | 20 % |

a: -, negative records indicate a stimulation of growth of the wheat rootlets.

TABLE 2

Inhibition of the growth of the rootlets of wheat by temperate plant extracts at 10% concentration.

Inhibición en el crecimiento de las raíces de los granos de trigo ejercida por extractos de plantas de zonas templadas, a una concentración del 10%.

| Plant: Scientific name and family | Percent inhibition |
|---|---|
| *Achyrocline flaccida D.C.* (Compositae) | 55 % |
| *Amaranthus quitensis Bompland et Kunth* (Amaranthaceae) | 98 % |
| *Ambrosia tenuifolia Spr.* (Compositae) | 100 % |
| *Aster squamatus (Spreng) Hieron.* (Compositae) | 100 % |
| *Baccharis coridifolia D.C.* (Compositae) | 99 % |
| *Baccharis grisebachii Hieron.* (Compositae) | 100 % |
| *Baccharis magellanica (Lam) Persoon.* (Compositae) | 100 % |
| *Baccharis tucumanensis H. et A.* (Compositae) | 98 % |
| *Balbisia calysina (Griseb) Hunz.et Ariza.* (Ledocarpaceae) | 95 % |
| *Bolax gummifera (Lam) Sprengel.* (Apiaceae) | 32 % |
| *Chiliotrichum diffusum (Forst.) OK.* (Compositae) | 91 % |
| *Empetrum rubrum Willd.* (Empetraceae) | 100 % |
| *Eupatorium arnottianum Griseb.* (Compositae) | 90 % |
| *Eupatorium buniifolium H.et A.* (Compositae) | 99 % |
| *Eupatorium candolleanum H.et A.* (Compositae) | 94 % |
| *Eupatorium christieanum Bak.* (Compositae) | 100 % |
| *Eupatorium hecathantum (D.C) Bak.* (Compositae) | 86 % |
| *Gamochaeta simplicicaulis Cabr.* (Compositae) | 94 % |
| *Gentianella achalensis Hieron. ex Grieb.* (Gentianaceae) | 92 % |
| *Parodiochloa flabellata L.* (Poaceae) | 79 % |
| *Phyllanthus sellowianus Muell. Arg.* (Euphorbiaceae) | 59 % |
| *Prosopis flexuosa De Candolle* (Leguminoseae) | 100 % |
| *Prosopis nigra (Gris.) Hieron.* (Leguminoseae) | 98 % |
| *Pterocaulon polystachium D.C.* (Compositae) | 100 % |
| *Pterocaulon purpurascens Malme.* (Compositae) | 98 % |
| *Satureja parvifolia Epling.* (Labiateae) | 67 % |
| *Satureja odora Epling.* (Labiateae) | 68 % |
| *Senecio candicans De Candolle* (Compositae) | 43 % |
| *Terminalia triflora (Griseb) Lillo* (Combretaceae) | 100 % |
| *Trifolium repens L.* (Leguminoseae) | 100 % |

ting effects. We found a significant difference in the proportion of species that caused allelopathic inhibition of wheat rootlet growth between the two plant communities ($p < 0.0001$). Significant differences in the proportion of plant species that stimulate the growth of the rootlets of wheat were also observed between both communities ($p<0.002$). The observed results are summarized in Figure 1. Figure 2 compares in an illustrative way the percentage inhibition of the growth of wheat rootlets for plants belonging to tropical or temperate communities.

## DISCUSSION

This paper reports the differences in allelopathic effects of plant species from tropical and temperate areas, tested by the method of the inhibition of the growth of the rootlets of

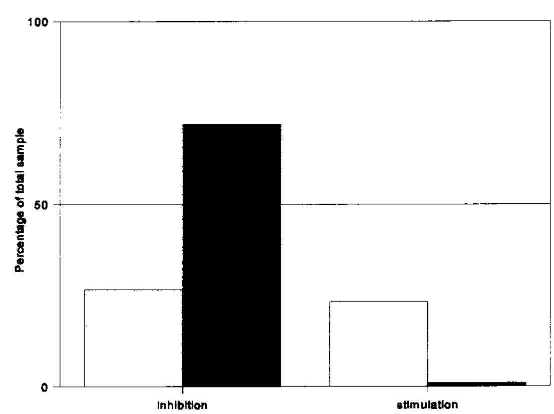


*Fig. 1:* Effects of tropical and temperate community plants extracts on the wheat rootlet growth inhibition test. □ Plants belonging to a neotropical community. ■ Plants belonging to a temperate community. Inhibition indicates the percentage of plants that exercise allelopathic effects (90% or more inhibition) on the wheat rootlet growth in both community samples. Stimulation indicates the percentage of plants that stimulate (negative (-) inhibition) the growth of the wheat rootlets in these samples.

Efectos de extractos de plantas provenientes de comunidades tropicales y templadas sobre el crecimiento de las raíces de los granos de trigo. □ Plantas pertenecientes a una comunidad neotropical. ■ Plantas pertenecientes a una comunidad templada. La inhibición indica el porcentaje de plantas que ejercen efectos alelopáticos (inhibición igual o mayor a 90%) en el crecimiento de las raíces de los granos de trigo. La estimulación indica el porcentaje de plantas que estimulan (inhibición negativa (-)) el crecimiento de las raíces de los granos de trigo.

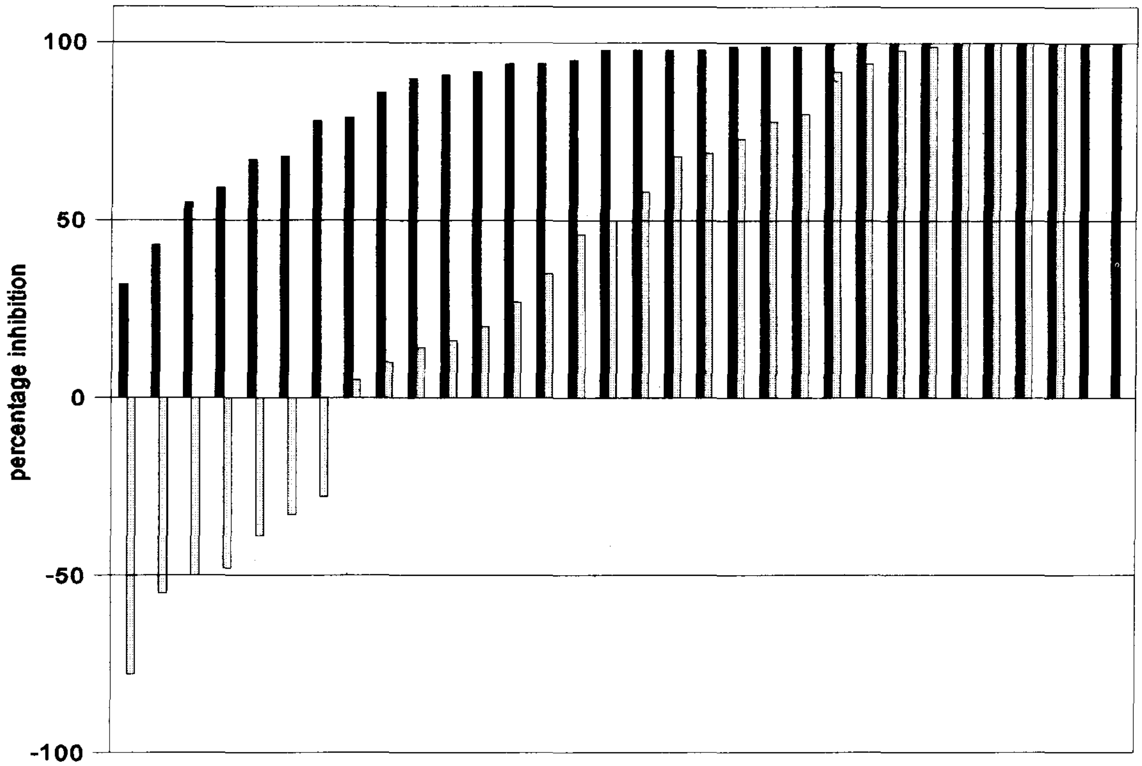


*Fig. 2:* Comparison of the percentage inhibition of plants extracts in the wheat rootlet growth inhibition test. □ Plants belonging to a tropical community. ■ Plants belonging to a temperate community. Negative (-) records for % inhibition indicate a stimulation in the growth of the wheat rootlets.

*Figura 2:* Comparación de los porcentajes de inhibición de extractos de plantas en el crecimiento de las raíces de los granos de trigo. □ Plantas pertenecientes a una comunidad neotropical. ■ Plantas pertenecientes a una comunidad templada. Los valores negativos (-) para el % de inhibición indican una estimulación en el crecimiento de las raíces de los granos de trigo.

wheat (Boily & Harelimana 1979). The use of in vitro systems is of particular value when a quantitative measure of specific response is required such as the inhibition of growth (Hance & McKone 1976), and may be a potential method for estimating field results. In this sense, in vitro models can be cosidered as good predictors of results to be obtained in in vivo systems. However, it is important to consider that in vitro models can also be limiting in that they do not consider that in wet environments (e.g., tropical rainforest) leachates from plant leaves will be washed off rapidly from the soil, and hence their potential phytotoxic effects may be greatly reduced. Furthermore, they do not take into account the possible influence of other factors such as soil structure and/or microflora. Finally, the concentrations studied may not necessarily correspond to the concentrations measured in the soil solution in the natural habitat.

Clearly, temperate plant species showed a stronger allelopathic effect on the radicular growth of wheat than tropical species, while plant species from a neotropical community proved to be able to even stimulate the rootlet growth in this study (Figures 1 and 2). If this behaviour proves to be true for other species different to wheat, it could help to explain the existence of equilibrating forces that maintain a particular mixture of plants in tropical forests compared to a less diverse assemblage structure in temperate areas. Just how this form of interaction among species evolved in yet unclear. However, it is now accepted that there is great variation in the capacity of plants for the production of allelochemicals, both among species and within a species.

Likewise, there is considerable diversity among plants in susceptibility to the allelochemicals. It is because of this variation in both production and sensitivity that allelopathy may influence patterns of plant distribution in time and space in natural communities, and through allelopathy some plants gain an advantage.

Allelopathic systems in tropical forests may have two very different effects. Webb, Tracey, and Haydock (1967) have shown that the roots of adult Grevillea robusta A. Cunn. trees release a compound that kills their own seedlings in Australian rain forests, leading to wide spacing of adults. However, this conspecific effect has not been described for any of the plants studied in this paper. While this behavior cannot lead to spacing of new adults much past the root territory of the parental tree, it certainly will affect the posdispersal predators, and this allelopathy may also serve as an effective escape mechanism from a very effective predispersal seed predator that has great difficulty moving between adults (Janzen 1970). On the other hand, the "allelopathic" activity of ants on ant-plants in killing other plants around the parent tree often aids in producing local pure stands of Cecropia, swollen-thorn acacias, Tachigalia, etc. (Janzen 1969). Future studies of these aspects could reinforce a model in which allelopathy could help explain the differences in plant interference existing in plant communities with different levels of diversity.

A major question that remains unanswered is why should highly diverse plant communities show less allelopathic activity among their components (species) than less diverse ones. The Janzen (1970) and Connell (1971) hypothesis suggests that as one moves from the wet lowland tropics to the dry tropics and to temperate zones, the seed and seedling predators in a habitat should be progressively less efficient at keeping one or a few tree species from monopolizing the habitat through competitive superiority. This lowered efficiency of the predators is brought about by the increased severity and unpredictability of the physical environment, which in turn leads to regular or erratic escape of large seed or seedling from the predators. Studies of the role of allelopathic effects in maintenance of different degrees of plant diversity are not available in the literature at hand. However, further research on conspecific allelopathic interactions could help explain this correlation between high plant diversity and allelopathy.

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