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The leaching of Eucalyptus hybrids and *Acacia auriculiformis* leaf litter: laboratory experiments on early decomposition and ecological implications in congolese tree plantations

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The leaching of Eucalyptus hybrids and *Acacia auriculiformis* leaf litter: laboratory experiments on early decomposition and ecological implications in congolese tree plantations

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Abstract

The first stage of leaf litter decomposition of two eucalyptus hybrids and *Acacia auriculiformis* was studied in laboratory experiments where the decaying leaves were leached with water. Although the litter of both the species were characterized by a high content of soluble compounds, little soluble organic matter of acacia litter was recovered in litter leachates. By contrast, soluble organic matter of eucalyptus litter was more resistant to degradation and was, therefore, recovered in leachates, where soluble carbon amounts and coloration varied with the age of decomposing litter. The pH of eucalyptus litter leachates decreased at the beginning of the experiment whereas the pH of acacia litter leachates increased. When added to savanna soil, litter leachates mobilized iron into soil solution, with greater effects observed from eucalyptus than from acacia. In bioassay, litter leachates were added to sand in which test plant seedlings were grown, and a strong allelopathic activity was observed with leachates from the first days of eucalyptus litter decomposition. Leachates from older litter did not show any effect. The possible ecological implications of the observed processes are discussed.

Keywords: Tree plantations; Litter decomposition; Soluble carbon; Iron mobilization; Allelopathy; Congo

1. Introduction

Exotic trees are widely planted in tropical countries for industrial purposes as well as for afforestation, and ecological implications of such practices are often questioned (Poore and Fries, 1985). Large scale eucalyptus plantations particularly fall under criticism as one of the most often reported effects is the suppression of associated vegetation. The allelopathic effect of soluble compounds in eucalyptus was reported by many authors (Del Moral and Muller, 1969; Al-Naïb and Al-Moussawi, 1976; Jayakumar et al., 1990; Lisanework and Michelsen, 1993). Another reported effect of eucalyptus is the trend toward podzolization that was observed by Enright (1978) in natural stands who related it to the ability of soluble organic matter from litter to mobilize iron in soil. Iron mobilization was also reported by Ellis (1971). The role of dissolved organic matter in acidification and podzolization is known in temperate forests (Bruckert et al., 1971).

Litter quality of Australian acacia has been less well investigated and most reports have been concerned with nutrient content (Zech and Kaupenjohann, 1990;

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Pakrashi, 1991. Dreschel et al. (1991) suggested that the low decomposition rate of *Acacia auriculiformis* was caused by its high tannin content. However a much lower tannin content in *A. auriculuformis* litter than in eucalyptus litter was measured by Bernhard-Reversat and Schwartz (1997).

The ecology of tree plantations was studied in the coastal region of Congo, where more than 40 000 ha of savanna are planted with eucalyptus, an important plantation tree in many African countries; as an alternative to eucalyptus, Australian acacias are widely planted in tropical Asia, and they are found in some experimental plantations in Congo.

It was previously shown that eucalyptus and *Acacia auriculiformis* leaf litter exhibited a high content of soluble organic matter, ranging from 30% to 35% of dry weight. Moreover it was demonstrated that weight loss from fresh litter was equal to the loss of soluble compounds during the first weeks of in situ decay (Bernhard-Reversat, 1993). This emphasized the dependence of early decomposition processes on soluble organic matter. High soluble organic matter content in eucalyptus litter was also reported for other eucalyptus species by Wood (1974), Bernhard-Reversat (1987) and O'Connel (1987). Therefore, the objectives were to study the early decomposition processes in laboratory experiments in order to assess the potential influence of leaf litter leachates on plantation soil.

2. Material and methods

The study was conducted in two eucalyptus hybrid, PF1 and 12 ABL × saligna (HS2), and *A. auriculiformis* plantations which were described in a previous paper (Bernhard-Reversat, 1993). The plantations are located near Pointe Noire (Congo). Mean annual precipitation is 1250 mm, with 4 dry months from June to September. Mean annual temperature is 25°C. The highly desaturated ferrallitic soil (Jamet and Rieffel, 1976) is unvaryingly sandy, with low levels of nutrients, especially nitrogen. The main purpose of the study was to compare tree stands to the native herbaceous savanna on which they were grown, dominated by *Loudetia togoensis* and *Ctenium newtonii.*

Although Australian acacia have phyllodes as photosynthetic organs, these are referred to as 'leaves' throughout this paper. Samples were recovered by collecting recently fallen leaf litter, identified by its yellow or light-brown color, or by collecting weekly litter-fall in wire net quadrats. The leaves were cut into pieces approximately 3–6 cm^2. in area. In laboratory experiments, 5–8 g of litter were laid on washed sand in PVC tubes 56 mm in diameter and 22 cm in length with a 100 mm mesh sieve at the bottom. Percolations with 60 ml of water were performed each day for 5 days and every 2 or 3 days for 2 or 3 further weeks. Leachates were analysed for carbon content, pH, and optical density at 472 and 665 mm, which are the wavelengths used for the characterization of phenolic compounds (Hanrion et al., 1975). Dissolved carbon was determined as DCO on 2 ml of leachate boiled for 2 h in sulphuric acid with $K_2Cr_2O_7$ and $AgCl_2$ added and titrated with Mohr salt and $KMnO_4$ (Reversat, 1981). After 21–30 days, litter samples were rapidly washed and dried for weight loss determination. Six assays were made successively with eucalyptus: one with PF1, HS2 and acacia (with two replications), three with PF1 and acacia (with four or five replications), one with PF1 and HS2 (with three replications) and one with PF1 alone (with six replications).

Soluble phenolics were measured from three replications of PF1 and HS2 eucalyptus litter on sand in one of the assays. Dissolved phenolics were measured in leachates with tyrosine reagent ($Hach^R$ reagents, Anon., 1994) which takes into account all hydroxyled aromatic compounds. The results were expressed as equivalent of tannic acid.

A further experiment, with litter laid on washed sand, was conducted as above for 14 days, for iron mobilization measurements. After each percolation, aliquots of 70 ml of leachates were shaken with 30 g of savanna soil, and centrifuged. Iron was determined colorimetrically in the solution after the organic matter was mineralized with H_2O_2. Controls were made with water percolated on sand without litter, and shaken with 30 g of soil. Three assays allowed comparisons of eucalyptus hybrids to be made with ANOVA. After the reproducibility of the measurements was checked, one assay was conducted with two replicates of percolations which were combined for iron extraction, allowing comparison of eucalyptus and acacia by means of a paired *t*-test.

Allelopathy was estimated through bioassay with the percolates of litter on washed sand from two of the previous assays. Two-day-old rice seedlings (*Oriza* *sativa*, var. Moroberekan) were planted on washed sand in 150 ml vessels and watered daily with 10 ml of litter leachates. Two replicate assays with 7–10 seedlings each were carried out for each treatment (age of litter and litter species). When they were 9-days-old, roots were recovered by washing, and the lengths of the four to seven main roots were measured for each seedling whereas the thin lateral roots were not measured. Shoots and roots were dried and weighed separately for each vessel. Root thickness was estimated as total root weight/root length ratio; the error made by including lateral roots in the root weight was negligible because they were very thin.

Thin cross-sections of brown leaves were observed under the microscope in order to observe the distribution of brown compounds in leaf litter.

The correlation coefficients and curves were calculated with simple regression models.

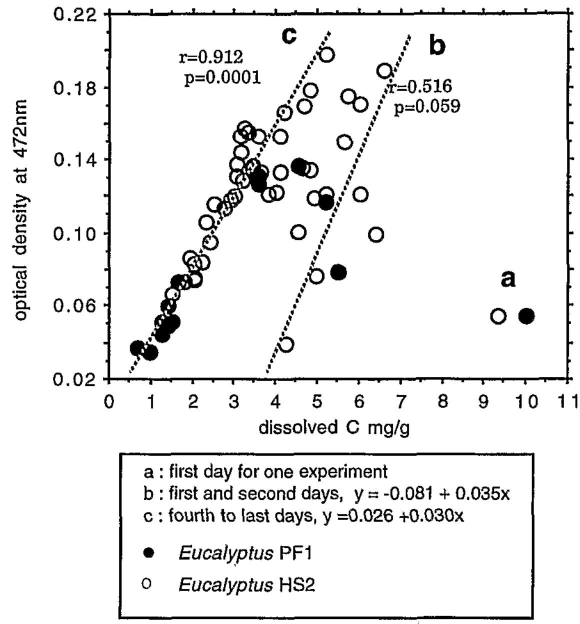

Fig. 1. Dissolved carbon versus optical density at 472 mm in leachates from *Eucalyptus* PF1 and *Eucalyptus* HS2 decomposing litter.

3. Results

3.1. Leaf and leachate coloration

Recently fallen leaves were yellow or light-brown, turning brown within a few days. Microscopic observation of light-brown eucalyptus leaves showed that brown pigments were located in the epidermis of the leaf vein, and around the vessels. The leaf blade was not colored. In eucalyptus dark-brown leaves, coloration occurred in the epidermis, and in and around the vessels; most of the parenchyma was not colored. In light-brown acacia leaves, two or three layers of parenchyma cells under the epidermis were brown, and no coloration was seen in the vessels; in dark-brown leaves, all the parenchyma was brown, and the vessels did not exhibit any coloration.

Leachates from acacia litter were very slightly colored, unlike leachates from eucalyptus litter which were brown. Optical density at 472 mm of eucalyptus leachates showed a maximum on the 3rd and 4th day. Correlation with dissolved carbon content (Fig. 1) was high between the 4th and the last day ($r = 0.91$, $p = 0.0001$). It was lower between the 1st and the 3rd day ($r = 0.52$, $p = 0.06$) because the 3rd day intermediate values were sometimes recorded. A sharp increase in optical density at 472 mm and 665 mm and a decrease in their ratio, together with the blackening of leachates, was observed when the percolation was performed on savanna soil compared to pure sand.

3.2. Soluble compound release in leachates and pH change

In most experiments on eucalyptus litter, the highest carbon recovery in leachates was observed on the 2nd or 3rd day (Fig. 2(A)). However, in one experiment, dissolved carbon was highest on the 1st day. After 5–6 days there was a sharp decrease in the amount of leached carbon and then a relatively steady loss of carbon occurred during the remaining period, at a higher rate in PF1 than in HS2. Dissolved carbon from acacia litter was lower and almost nil after 6 days. The resulting balance of carbon is given in Table 1 for some experiments.

The phenolic compound content of leachates was greatest from the 3rd to the 5th day according to the assay, and was correlated with optical density at 472 and 665 nm ($p = 0.05$ and 0.04, respectively) but was more strongly correlated with optical density at 420 nm ($p = 0.0001$). It showed different correlations according to the incubation time (Fig. 3).

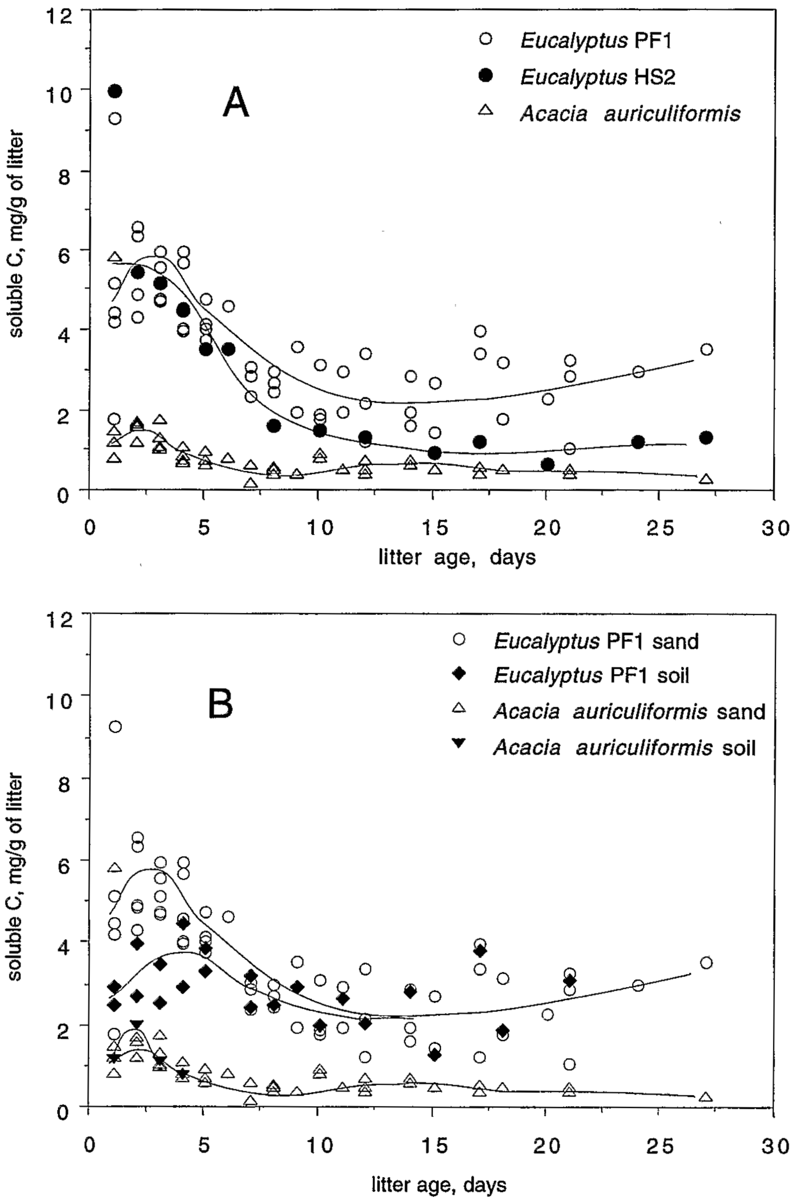

Fig. 2. Soluble carbon release by the decomposing litter of eucalyptus hybrids and *A. auriculuformis*. Curves were empirically adjusted and indicate trends. (A) Results of five assays with litter on washed sand. (B) Comparison of washed sand and soil as litter substrate.

A decrease in pH was observed in eucalyptus leachates with minimum values of 4.4–4.9. Acacia leachates pH increased slightly (Fig. 4). The average pH values for eucalyptus PF1 and HS2 were 4.83 and 4.91, respectively, the difference being just significant ($p = 0.05$, t-test of paired comparison). The difference between acacia (average 6.68) and eucalyptus was highly significant. A strong negative correlation was observed between pH and coloration at O.D. 472 ($r = -0.51$, $p = 0.0003$ for PF1, $r = -0.87$, $p = 0.0001$ for HS2)

Table 1
Organic matter (O.M.) fractions in five assays of litter decomposition on washed sand (and standard error)

Tree species or hybrid	*Eucalyptus*			*Acacia auriculiformis*	
	PF1	PF1	HS2		
Duration, days	27	21	27	27	21
Replications	2	5	2	2	5
Total weight loss %d.w.	28.7 (0.1)	25.7 (0.4)	22.2 (0.7)	23.3 (0.7)	22.9 (0.1)
Initial soluble O.M. %d.w.	34	27	30	30	23
Final soluble O.M. %d.w.	7.1 (0.5)	3.7 (0.01)	5.1 (0.4)	2.2 (0.02)	2.1 (0.01)
Soluble O.M. loss %d.w.	27	23	26	28	21
Leached soluble O.M. %d.w.	15.0 (0.1)	10.1 (0.4)	10.6 (0.6)	3.7 (0.05)	1.8 (0.02)
Leached % soluble O.M. loss	56	44	40	13	9

d.w. – initial litter dry weight.

3.3. Iron mobilization ability

Comparison of iron content in leachates from litter and control treatment indicated iron mobilization by eucalyptus, which was greatest between the 5th and 7th day (Fig. 5(A)). Iron mobilization by acacia litter occurred also but was weak. The overall data showed a significant difference between the iron content of eucalyptus leachates and the control ($p = 0.001$), between acacia and control ($p = 0.005$) and between eucalyptus and acacia litters ($p = 0.02$). A close relationship was observed between the iron content and pH, ($r = 0.83$, $p = 0.0001$) and specific correlations were also significant, except for control data (Fig. 5(C)).

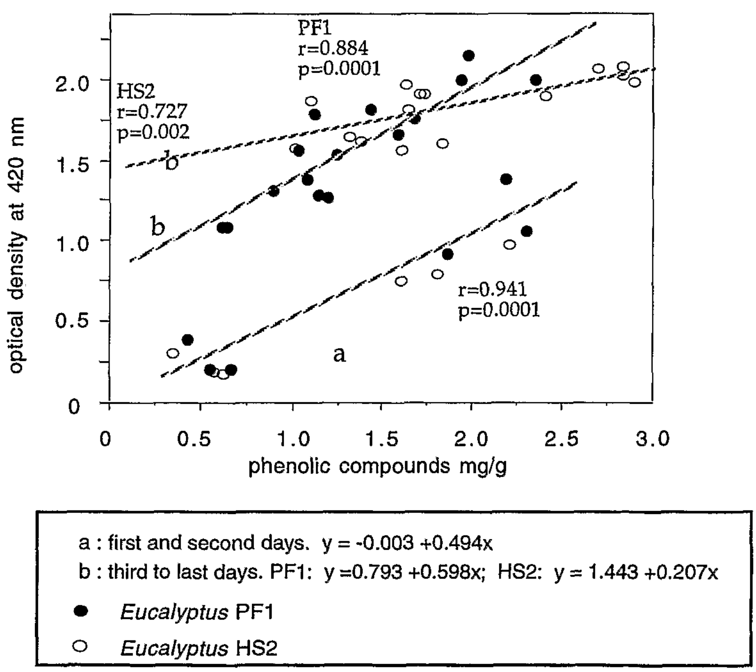

Fig. 3. Phenolic compound content versus optical density at 420 mm in leachates from *Eucalyptus* PF1 and *Eucalyptus* HS2 decomposing litter.

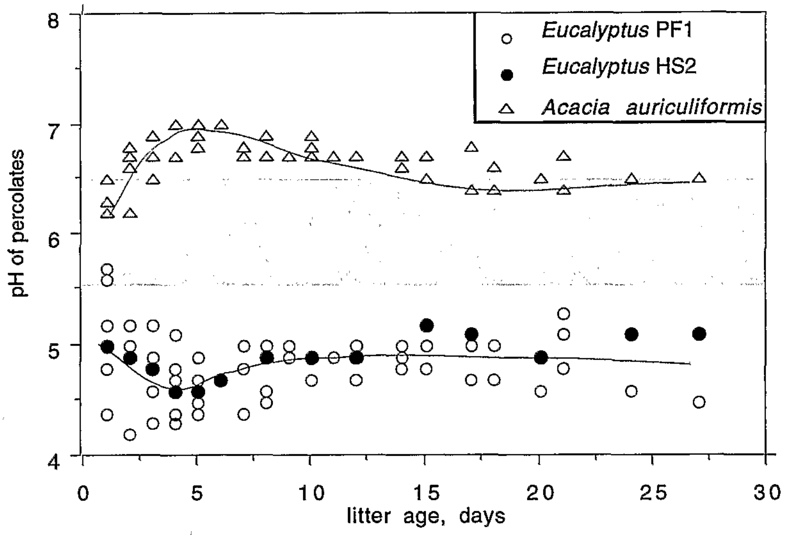

Fig. 4. Evolution of pH in eucalyptus and *A. auriculuformis* litter leachates from five assays with litter on washed sand. Curves were empirically adjusted and indicate trends.

When the various assays were considered, a difference in iron extraction ability was observed between the two hybrids of eucalyptus ($p = 0.003$, Fig. 5(B))

3.4. Allelopathic effect

The application of eucalyptus leachates to rice seedlings resulted in a sharp decrease in root length together with an increase in root weight/root length ratio (Fig. 6) and a decrease in the number of lateral roots. These effects lasted a few days and leachates from older litter had no effect. Root measurements were correlated with the dissolved carbon content of leachates, with $r = -0.86, p = 0.0003$ for root length, and $r = 0.80$, $p = 0.003$ for root weight/root length ratio. Acacia leacheates showed a weak effect, on root weight/root length ratio only.

4. Discussion

4.1. Leaf coloration

According to Toutain (1987) leaf brown pigments originate from vacuole phenolic compounds mixed with cytoplasmic proteins and are resistant to degradation. The parenchyma coloration of acacia brown leaves and the low solubility of their pigments are in agreement with the general trend described by this author. However, eucalyptus exhibited a peculiar behaviour and most of the parenchyma of brown leaves was not colored. The occurrence of coloration near and in the vessels appeared to be linked to readily-leached, colored compounds.

4.2. Soluble carbon release

The carbon balance of the experiment suggested that most weight loss was due to soluble organic matter loss, as previously described (Bernhard-Reversat, 1993). Some discrepancy which occurred in some data might be related to the lack of accuracy in weight loss measurements. The results emphasized the weak recovery of soluble compounds in leachates from acacia litter. Leachates from acacia were previously demonstrated to be rapidly mineralised (Bernhard-Reversat, 1993). Therefore, the low level of soluble carbon in litter leachates from acacia during the present experiments might result from its mineralisation during incubation. Upto 40% or 50% of soluble compounds were recovered from the eucalyptus litter, (accounting for 10–15% of initial litter dry weight). In a 6 week in situ experiment with oak litter, Tietema and Wessel (1994) recovered only 21% of weight loss in dissolved organic compounds and 64% were mineralized through microbial respiration. Swift et al.

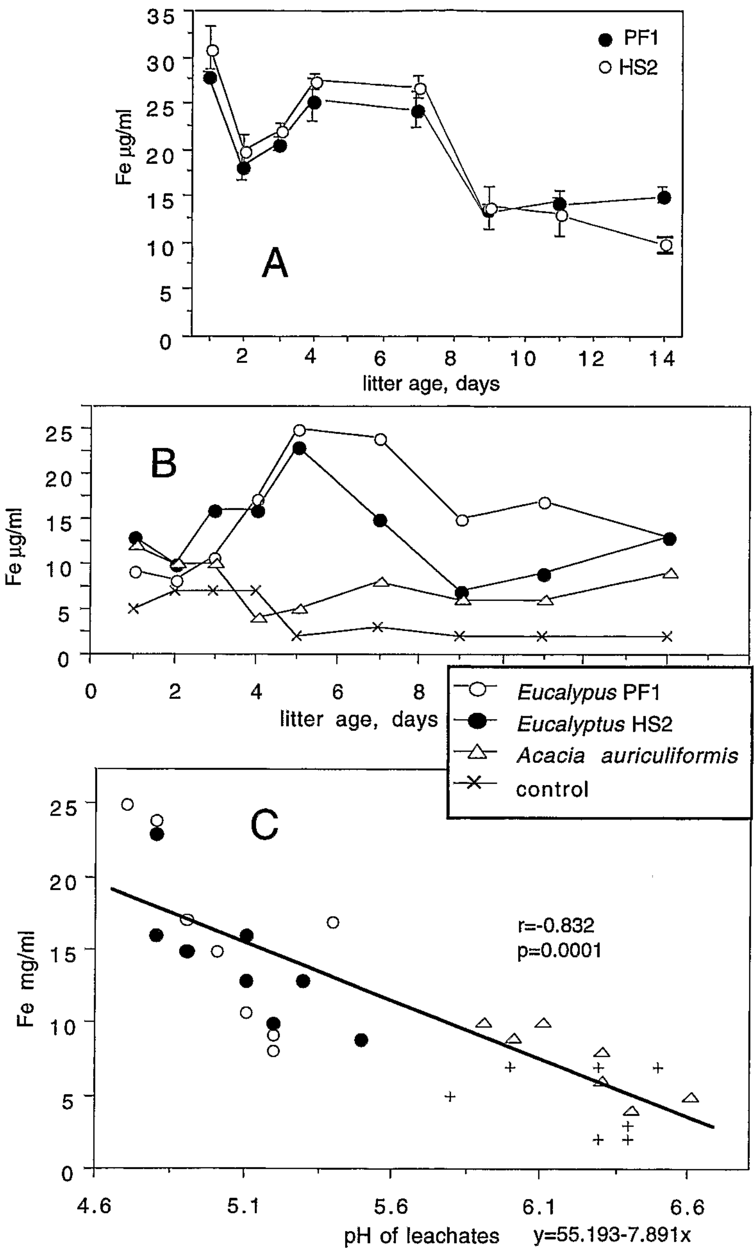

Fig. 5. Iron release by soil in eucalyptus and *A. auriculuformis* litter leachates during early decomposition. (A) Change in iron content of leachates from eucalyptus litter (three replications). (B) Change in iron content from eucalyptus and acacia litter. (C) Relationships of iron content with leachate pH.

(1979) reported that leaching is a component of weight loss which is rarely quantified, and until now few data were available. *A. auriculiformis* leachates are assumed to have little effect, as most of the dissolved carbon was readily mineralised, resulting in possible losses of about 30% of litter-fall. Unlike acacia, eucalyptus litter released a great amount of soluble carbon to the soil.

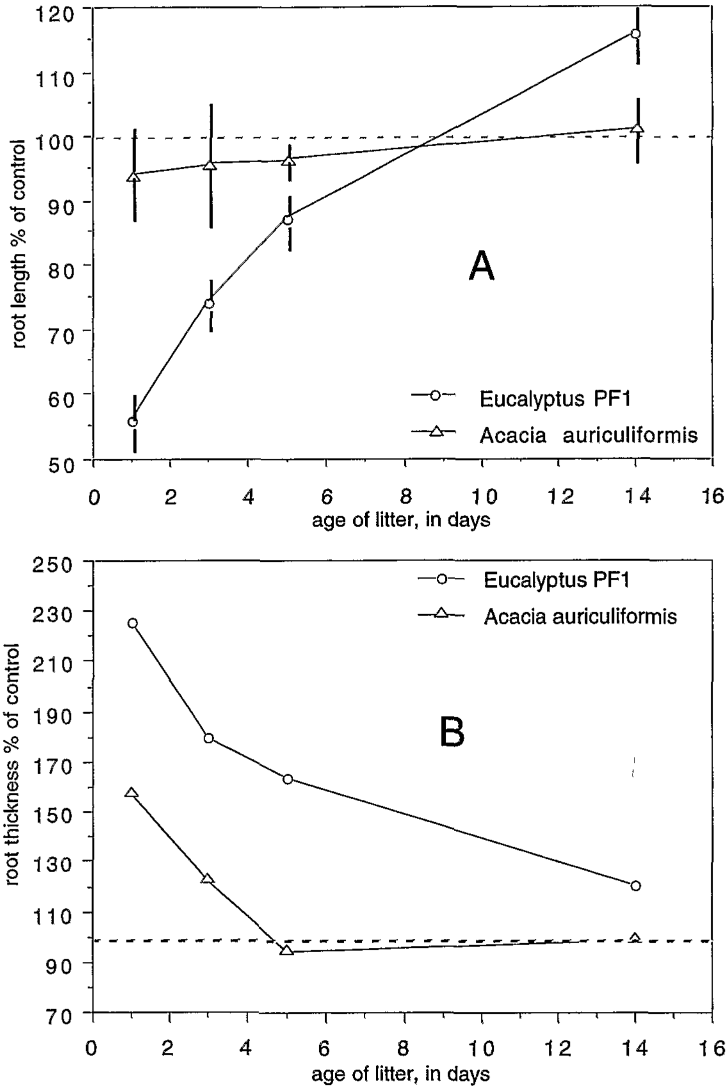

Fig. 6. Allelopathic effect of *Eucalyptus* and *A. auriculuformis* litter leachates during early decomposition. (A) On root length. (B) On root thickness.

A slow biodegradation of organic matter from throughfall and forest floor leachates in oak forest was reported by Qualls and Haines (1992), and polyphenol content of leachates was thought to be responsible for the low mineralisation rate of soluble carbon (Basaraba and Starkey, 1966). High amounts of tannins were found in the eucalyptus litter where it reached 4% of litter dry weight, whereas only 0.75% was found in acacia litter (Bernhard-Reversat and Schwartz, 1997).

The high amount of dissolved carbon which was observed once on the 1st day in eucalyptus litter (Fig. 1) might correspond to a readily leached pool of soluble carbon, which may have been already leached from other samples by rain prior to litter collection. This initial pool, observed only once in the rainy conditions of Congo, was often observed in similar experiments under a dry climate (Senegal) with *Eucalyptus calmadulensis* (Bernhard-Reversat, 1987). A second pool which could correspond to the second day maximum, and a third pool which could correspond to the relatively steady release occurring after a sharp decrease on the 4th and the 6th day, are suggested by the relationships between soluble carbon or phenolic compounds and optical density (Fig. 2). Whether these pools come from different parts of the leaf requires additional anatomical investigations on decomposing leaves.

Wershaw et al. (1996) employed very extensive fractionation methods for dissolved carbon in litter leachates and separated various fractions thought to represent different stages in the oxidative degradation of lignin and other aromatic components of the leaves. However, the short duration of our experiments suggests that mainly soluble compounds are recovered, as lignin degradation was shown not to occur in unfragmented eucalyptus litter (Bernhard-Reversat and Schwartz, 1997).

According to Swift et al. (1979), decreasing pH in decaying litter is mainly due to the leaching of acidic material from the vacuoles. For Qualls and Haines (1991), most humic and humic-like substances are found in the acidic fraction of dissolved organic matter in waters from Quercus forest, in agreement with the relationship between pH and optical density found in eucalyptus leachates. Although a decrease of soil pH by 0.5 to 1 unit was reported by Loubelo (1990) and Bandzouzi (1993) in the top layer of 7–11 years old eucalyptus plantations, soil pH was lower under acacia than under eucalyptus (Mboukou-Kimbatsa et al., 1998), and the main effect of the low pH of eucalyptus litter extracts is more likely to be expected through cation leaching rather than in soil acidification.

4.3. Activity of leachates

Although the effects of soluble carbon release from freshly fallen leaf litter are of short duration, they may be significant as litter-fall occurs throughout the year.

Soluble iron contents in leachates mixed with soil showed the efficiency of iron mobilization by eucalyptus. Besides, the sharp increase of optical density at 472 and 665 mm, combined with the darkening of leachates which were observed when litter was percolated on soil compared to sand might be attributed to the composition of the phenolic compounds-ferric iron complex (Kimura and Wada, 1989).

In oak and pine forests Malcolm and McCracken (1968) observed that phenolic compounds, organic acids and reducing sugar were responsible for iron mobilization by canopy drip, and Pohlman and McColl (1988) showed that iron mobilization by litter extracts was strongly correlated with organic acids and phenolic compounds. The high phenolic content of eucalyptus and its low level in acacia litter could be responsible for their different ability to mobilize iron. Organic acids were not measured in this study, and Fig. 5(C) shows that the ability to mobilize iron was also related to the pH of percolates.

It is difficult to compare the amounts of mobilized iron with other studies as these amounts reflect on experimental methods and conditions. Iron mobilization is closely related to soil mineralogy, particularly through the occurrence and activity of iron oxides in the clay fraction. Iron mobilization by eucalyptus was studied by Ellis (1971). The significance of the iron mobilization ability of litters for soil changes was underlined by Malcolm and McCracken (1968) and Enright (1978) correlated it with podzolization processes. Ellis (1971) reported that inter-specific and intra-specific variations in iron mobilizing activity of eucalyptus leaf and litter extracts were inversely related to soil fertility and that eucalyptus grown on poor soils were more likely to generate podzolization; in poor soils he observed a decrease in the iron content of the top soil layer. Surface soils of the studied region are impoverished in iron, which ranges from 1.5% to 4.5% (Jamet and Rieffel, 1976). Its distribution in soils of the congolese plantations was not studied but iron mobilization and leaching might be expected as the soil was very nutrient poor. The susceptibility of the soils of the coastal region of Congo to podzolization was reported by Schwartz and Guillet (1990) who observed podzols under 50–150-year-old forests which had encroached on savanna. However visual signs of podzolization were not conspicuous in soil profiles of the plantations studied.

The allelopathic effect of eucalyptus has been demonstrated with test plants by numerous authors among them Del Moral and Muller (1969), Al-Mousawi and Al-Naïb (1975), Igboanugo (1988), Molina et al. (1991) and Lisanework and Michelsen (1993). Litter leachates during initial decomposition affected mainly root growth of rice seedlings and no significant effect was measured on aerial parts. Roots were shorter and thicker than in the control, and less and shorter lateral rootlets were observed. As might be expected, this effect was correlated with the carbon content of leachates (Fig. 6(C)). As shown in Fig. 6((A)(B)), this was a very transient process, and leachates from litter older than 5 days did not exhibit any effect. There was some discrepancy with the results of Molina et al. (1991) who reported that decomposing litter of *Eucalyptus globulus* expressed a maximum allelopathic effect at 30 days and no effect after 45 days, but the absence of earlier measurements precluded observation of a more immediate effect. Previous results showed a drastic inhibition of root growth by powdered fresh litter and fresh litter extracts, while no effect was observed with old litter (Bernhard-Reversat, 1991). This short duration might be attributed to the solubility and leaching of the compounds responsible which could be low molecular weight phenolics (Harborne, 1997) rather than to their biodegradation, as allelopathic effects were recorded in top soil under eucalyptus (Molina et al., 1991, and the author's unpublished results). Litter-fall occurs throughout the year in eucalyptus plantations (Bernhard-Reversat, 1993) and allelopathic compounds are likely to be leached from fresh litter during the entire rainy season (Molina et al., 1991). Sanginga and Swift (1992) suggest that the decreasing effect of eucalyptus litter on plant growth could also be due to nutrient immobilization because of the high C/N ratio of this litter. However the lack of effect of percolates on aerial parts and the absence of effect when old litter is added suggest an allelopathic effect rather than poor nutrient relations in this study.

Allelopathy was studied with a test plant which may not react in the same way as native vegetation; however, allelopathy in the field was also suggested by the scarce understory vegetation in most of the 3–7-year-old plantations plots after the herbaceous species, which tend to invade the plantations, have been weeded during the first 2 years, and when litter-fall was well established. The shade under those eucalyptus is minimal and cannot explain the lack of understory vegetation. Other factors such as tree root density or water and nutrient competition could be involved. Woody species grow back in second rotation plantations after tree logging and exploitation, when litter-fall is low, and they could be less sensitive to allelopathy than herbaceous plants when they are established. Field experiments are necessary to confirm the occurrence of allelopathic processes.

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