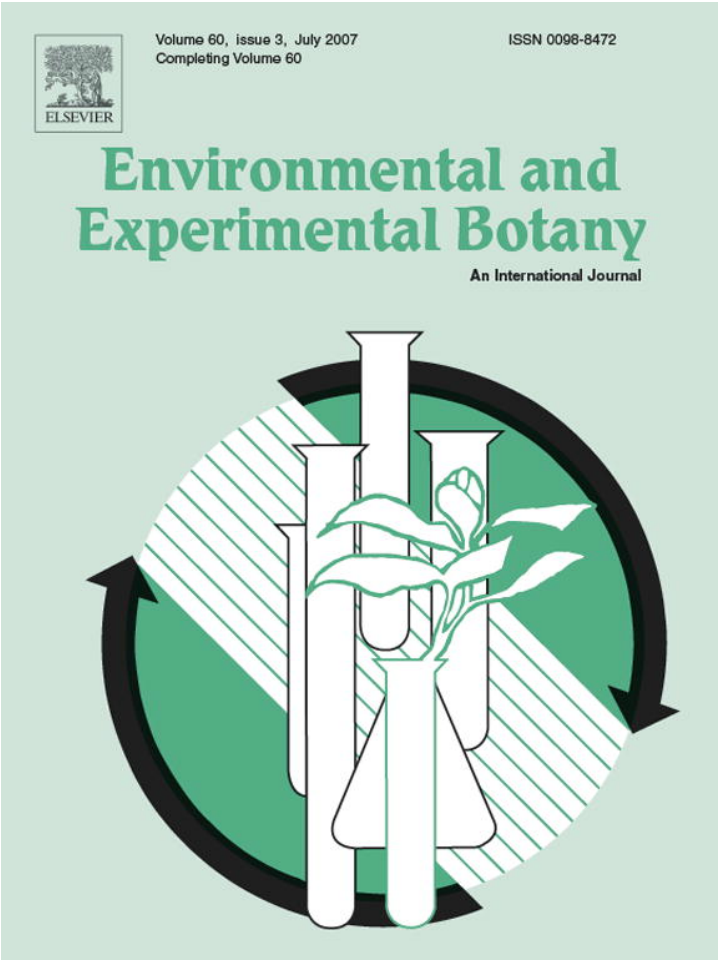

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Role of sugarcane straw allelochemicals in the growth suppression of arrowleaf sida

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Abstract

Previous studies suggested that allelochemicals from sugarcane straw may suppress the growth of arrowleaf sida (*Sida rhombifolia* L.). A study was conducted to establish: (1) the direct or indirect role of the organic molecules from sugarcane straw leachate on the growth suppression of arrowleaf sida and (2) if leachate phytotoxins induce proline accumulation in arrowleaf sida tissues as an adaptative response to a water or an oxidative stress. Inhibition of root elongation was the primary effect of sugarcane straw leachate on arrowleaf sida grown in unsterile soil. Addition of activated charcoal to unsterile soil before incorporation of straw leachate reduced the inhibition in root growth suggesting a direct participation of organic molecules in leachate phytotoxicity on arrowleaf sida. Inorganic straw constituents did not inhibit root growth while microbial activity increased leachate phytotoxicity. Soil chemical analysis suggested a direct action of organic molecules in leachate phytotoxicity rather than variations in macro and micronutrients or nutrient microbial immobilization. Straw leachate induced proline accumulation in roots and cotyledons of arrowleaf sida. Proline increase was related with oxidative stress in the roots but not in the cotyledons. Our results indicate a direct action of organic compounds from sugarcane straw and/or their microbial transformation products on root growth of arrowleaf sida. These substances induced proline accumulation in roots mainly as consequence of an oxidative stress while water stress may be the main cause of high proline content in the cotyledons. Although the observed responses could be due to phenolic compounds, the involvement of organic molecules with other chemical nature could not be excluded.

Keywords: Arrowleaf sida; Oxidative stress; Phenolic compounds; Sugarcane straw; Water stress

1. Introduction

Allelopathy appears to be an important component of plant interference capability in a variety of natural and managed ecosystems (Weston and Duke, 2003). Several authors have been hypothesized that allelopathic characteristics might be exploited for weed control purposes in a variety of agricultural settings (Singh et al., 2003). Many crop plants may provide toxicity to weeds upon decay of their residues (Chou, 1999), being possible to use this characteristic to reduce the destructive effects of current cultural practices and high energy inputs into agroecosystems (Singh et al., 2003). In Argentina and other countries, retention of postharvest sugarcane straw on the soil surface showed to reduce weed biomass (Sampietro, 2006). Previous research suggested that organic molecules from the straw leachate inhibit the growth of beggarticks (*Bidens subalternans* L.) and wild mustard (*Brassica campestris* L.) (Sampietro and Vattuone, 2006a), being phenolics the responsible growth inhibitors (Sampietro and Vattuone, 2006b; Sampietro et al., 2006). Sugarcane straw leachate induced foliar proline accumulation, a characteristic physiological response from higher plants to several environmental stresses (Szekely, 2004). In plants, proline accumulation has been widely related to osmotic adjustment necessary to overcome water stress. Several authors have suggested that proline could also protect cellular functions against free radicals when plants are subjected to oxidative stress (Bohnert and Shen, 1999). Oxidative stress related with weed exposition to sugarcane straw leachate was not previously assessed and the cause of proline accumulation in the exposed weeds remains obscure (Sampietro and Vattuone, 2006a,b).

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Arrowleaf sida is one of the most important broad-leafed weeds of the sugarcane fields in Brazil and Argentina (Lorenzi et al., 1989). This weed dissapears when straw is retained on soil surface (Manechini, 2000; Sampietro et al., 2006). Suppression of arrowleaf sida is suspected to be a consequence of the release of straw allelochemicals (Lorenzi et al., 1989; Sampietro et al., 2006). Straw constituents, however, could directly affect physiological processes of arrowleaf sida or indirectly inhibit seedling growth through modifications of soil characteristics (Inderjit and Weiner, 2001; Sampietro and Vattuone, 2006a).

The purposes of the present study were: (1) to establish the direct or indirect role of the organic molecules from straw leachate on arrowleaf sida seedling growth and (2) to determine if leachate phytotoxins are able to induce proline accumulation in arrowleaf sida tissues and if this situation is an adaptative response to a water or an oxidative stress.

2. Materials and methods

2.1. Soil and plant materials

Soil (loam typic argiudol) from 0–10 cm depth was collected from a sugarcane field near San Pablo (26°52′ S, 65°19′ W), Tucumán, Argentina. Soil was air-dried at room temperature, sieved (2-mm sieve), and stored in paper bags. Sugarcane (*Saccharum officinarum* L.) straw var Tuc (CP) 77-42 was collected from a sugarcane field near San Pablo, one week after harvest. Straw was dried at 60 °C for 48 h in a forced dry oven and used for leachate assays.

2.2. Phytotoxicity of sugarcane straw leachate

2.2.1. Assays in unsterile soil

Preliminary observations showed that sugarcane straw was not uniformly distributed on soil after harvest. Densities observed in the field were between 765 and 83 g of dry straw m^{-2}. Accordingly, different amounts of dry sugarcane straw (563, 396, 167 and 62 g) were soaked in 8.8 L of double-distilled-water (hereafter referred as water) for 4 h, followed by filtration. The obtained leachates were sterilized by passing through sterile filter membranes (Millipore, 0.22 μm) and assayed. The filtered volumes were identified as T1 (64 g dry straw L^{-1}), T2 (45 g dry straw L^{-1}), T3 (19 g dry straw L^{-1}) and T4 (7 g dry straw L^{-1}) which corresponded to 765, 538, 227 and 83 g of dry straw m^{-2}, respectively. Plastic containers (40 cm × 46 cm × 18 cm) were used in bioassays according with Sampietro and Vattuone (2006a). In each container, unsterile (unautoclaved) soil (4 kg) was placed and 2.2 L of each leachate (T1, T2, T3 or T4) or water was added to reach the water field capacity. After that, 450 pregerminated seeds with uniform root length (1 mm) of arrowleaf sida (*Sida rhombifolia* L.) were uniformly sown at 2 mm from the container soil surface. Experiments were conducted in a greenhouse with day and night temperatures between 20–28 °C and at a 10 h photoperiod of natural light. Each treatment was replicated four times and the experiments were once repeated. Data of root and shoot length were collected 7 days after seed sowing. Percentage of inhibition of root elongation (PIRE) in unsterile soil was calculated as:

$$\text{PIRE} = \left[1 - \frac{\text{root length in unsterile soil treated with a given concentration of straw leachate}}{\text{root length in unsterile soil treated with water}}\right] \times 100$$

2.2.2. Residual effect of straw leachate

Assays were performed in plastic containers to study the residence time of sugarcane straw leachate in soil in terms of seedling growth inhibition. In each container (40 cm × 46 cm × 18 cm), unsterile soil (4 kg) was placed and 2.2 L of a straw leachate (T1) or water was added to reach the water field capacity. Pregerminated seeds of arrowleaf sida were sown, as previously indicated, in the containers at different dates (day 0, 1, 2, 3, 7 and 10) which corresponded to 0, 1, 2, 3, 7 and 10 days after T1 incorporation to unsterile soil. Water controls were also sown at the same sowing times. Greenhouse conditions were similar to those previously outlined. Each treatment was replicated four times and the experiments were once repeated. Data of root and shoot length were collected 7 days after seed sowing. Percentage of inhibition of root elongation (PIRE) in unsterile soil was calculated, for each date, as indicated above.

2.2.3. Assays in unsterile soil mixed with activated charcoal

Assays were performed in containers to determine the modification of sugarcane straw leachate phytotoxicity in unsterile soil after addition of activated charcoal. These substance can adsorb organic phytotoxins incorporated to unsterile soil, and is recommended to separate toxicity of organic molecules from other mechanisms of interference (Sampietro and Vattuone, 2006a; Wardle and Nilsson, 1997). In each container (40 cm × 46 cm × 18 cm), unsterile soil (4 kg) mixed with 15 g of activated charcoal (Sigma, USA) was amended with 2.2 L of each straw leachate (T1, T2, T3 or T4) or water. Pregerminated seeds of arrowleaf sida were sown in the containers as previously indicated. Greenhouse conditions were similar to those previously outlined. Each treatment was replicated four times and the experiments were once repeated. Data on root and shoot length were collected 7 days after seed sowing. Percentage of inhibition of root elongation (PIRE) in unsterile soil mixed with charcoal was calculated as:

$$\text{PIRE} = \left[1 - \frac{\text{root length in unsterile soil mixed with charcoal treated with a given concentration of straw leachate}}{\text{root length in unsterile soil mixed with charcoal treated with water}}\right] \times 100$$

2.3. Phytotoxicity of leachates from ashes of sugarcane straw

Assays were performed in containers to determine the phytotoxicity of inorganic straw constituents in unsterile soil. Dry amounts of sugarcane straw equal to those used to prepare the straw leachates (563, 396, 167 and 62 g) were completely burned. The obtained white ashes (65, 46, 19 and 5 g, respectively) were soaked in 8.8 L of water for 4 h, followed by filtration. The filtered volumes were identified as TS1 (7 g of straw ashes L^{-1}), TS2 (5 g of straw ashes L^{-1}), TS3 (2 g of straw ashes L^{-1}) and TS4 (0.6 g of straw ashes L^{-1}). Unsterile soil (4 kg) was placed in each container (40 cm × 46 cm × 18 cm) and amended with 2.2 L of leachate from ashes of sugarcane straw (TS1, TS2, TS3 or TS4) or water. Pregerminated seeds of arrowleaf sida were sown in the containers as previously indicated. Greenhouse conditions were those previously outlined. Each treatment was replicated four times and the experiments were once repeated. Data on root and shoot length were collected 7 days after seed sowing. Percentage of inhibition of root elongation (PIRE) in unsterile soil after incorporation of leachate from straw ashes was calculated as:

$$\text{PIRE} = \left[1 - \frac{\text{root length in unsterile soil treated with a given concentration of leachate from straw ashes}}{\text{root length in unsterile soil treated with water}}\right] \times 100$$

2.4. Analysis of unsterile soil amended with straw leachate and leachate of straw ashes

Soil samples treated with straw leachate and leachate of straw ashes were collected to establish possible modifications in soil properties that could indirectly inhibit arrowleaf sida growth. The samples were analyzed for the following soil characteristics: pH, organic matter, exchangeable PO_4^{3-}, soluble salts, Ca^{2+}, Mg^{2+}, K^+, Na^+, NO_3^-, NH_4^+, Mn^{2+}, Zn^{2+}, Fe^{3+} and total phenolics. Each treatment was replicated four times. Experiments were once repeated. Soil chemical analysis were performed using the following methods: pH in water (1:5 w/v), soluble salts in soil paste, organic matter (Walkley and Black, 1934), P by Kurtz and Bray method, N by Kjeldhal digestion, NO_3^- by Devardaĭs alloy method (Faithfull, 2002), NH_4^+ in MgO destilation and Ca^{2+}, Mg^{2+}, K^+ and Na^+ in ammonium acetate (pH 7) and available Fe^{3+}, Zn^{2+} and Mn^{2+} using an atomic absorption spectrophotometer (Allen, 1989). To determine total phenolics concentration, soil samples (5 g) were soaked and shaken with 20 mL of water and then filtered. Phenolics concentration was determined using the Folin–Ciocalteu phenol reagent (Swain and Hillis, 1959). Total phenolics are expressed as μg equivalents of ferulic acid. Each treatment was replicated four times and the experiments were once repeated.

2.5. Measurement of proline content

To estimate proline concentration, roots and cotyledons samples from 7 day-old seedlings of arrowleaf sida grown in unsterile soil treated with straw leachate (T1, T2, T3 or T4), in unsterile soil mixed with activated charcoal treated with straw leachate and in unsterile soil treated with leachate of straw ashes (TS1, TS2, TS3, TS4) were harvested. Seedlings grown in unsterile soil treated with water and unsterile soil plus charcoal treated with water were also grown and harvested. Each sample (0.5 g, fresh weight) was homogenized in aqueous sulfosalicylic acid (3%, p/v) and filtered with filter paper (Whatman #1). The reaction mixture consisted of 2 mL of filtered solution, 2 mL of acid ninhydrin (1.2 g of ninhydrin in 30 mL glacial acetic acid plus 20 mL of 6 M orthophosphoric acid) and 2 mL of glacial acetic acid, and was boiled at 100 °C for 1 h. Then, the reaction mixture was extracted with toluene (4 mL) and optical density was measured at 520 nm (Bates et al., 1973). Each treatment was replicated four times and the experiments were once repeated.

2.6. Measurement of oxidative stress

Oxidative stress was measured by mean of GPX activity and lipid peroxydation.

2.6.1. Measurement of guaiacol peroxidase (GPX) activity

To determine GPX activity, root and cotyledons samples from 7 day-old seedlings of arrowleaf sida grown in unsterile soil treated with straw leachate (T1, T2, T3 or T4), in unsterile soil mixed with activated charcoal treated with straw leachate and in unsterile soil treated with leachate of straw ashes (TS1, TS2, TS3, TS4) were harvested. Each sample (0.5 g, fresh weight) was ground in 2 mL of enzyme extraction buffer (composition: 0.5% (w:v) polyvinylpyrrolidone (PVP), 3 mM EDTA, and 0.1 M, pH 7.5 potassium phosphate buffer) along with a small amount of glass beads using a cold mortar and pestle (kept on ice). The samples were centrifuged at 3000 × *g* for 10 min at 2–5 °C, and then kept on ice. GPX activity was determined in a reaction mixture containing 0.1 M potassium phosphate buffer (pH 6.8), 56 mM guaiacol solution, 0.2 mM hydrogen peroxide and enzyme in a total volume of 1 mL. The oxidation of guaiacol was followed by monitoring the increase in absorbance (of the guaiacol polymer) at 470 nm ($\varepsilon_{470} = 26.6\ \text{mM}^{-1}\ \text{cm}^{-1}$) over a period of 5 min (McCue et al., 2000). Each treatment was replicated four times and the experiments were once repeated.

2.6.2. Measurement of lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Hodges et al. (1999). MDA is a product of lipid peroxidation and can be assessed through its reaction with thiobarbituric acid (TBA). Root and cotyledon tissue samples (0.5 g, fresh weight) from 7 day-old seedlings of arrowleaf sida grown in unsterile soil treated with straw leachate (T1, T2, T3 or T4), in unsterile soil mixed with activated charcoal treated with straw leachate and in unsterile soil treated with leachate of straw ashes (TS1, TS2, TS3, TS4) were harvested. Each sample was homogeinized in 3 mL of 80:20 (v/v) ethanol:water. The sample extracts were centrifuged at 3000 × *g* for 15 min at 4 °C. Then, 1 mL aliquot of each supernatant was added to a test tube with 1 mL of either

(1) a solution without TBA (−TBA) that was comprised of 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxitoluene, or (2) a solution containing the above plus 0.65% TBA (+TBA). Samples were then mixed vigorously, heated at 95 °C for 25 min, cooled and centrifuged at 3000 × g for 10 min. Absorbance was read at 440, 532 and 600 nm. Malondialdehyde equivalents were calculated in the following manner:

$$A = [(\text{Abs}\,532_{+\text{TBA}} - \text{Abs}\,600_{+\text{TBA}}) - (\text{Abs}\,532_{-\text{TBA}} - \text{Abs}\,600_{-\text{TBA}})] \quad (1)$$

$$B = [(\text{Abs}\,440_{+\text{TBA}} - \text{Abs}\,600_{+\text{TBA}})0.0571] \quad (2)$$

$$\text{MDA equivalents (nmol ml}^{-1}) = \left(\frac{A - B}{157,000}\right) \times 10^6 \quad (3)$$

Each treatment was replicated four times and the experiments were once repeated.

2.7. Data analysis

Experiments were conducted using a completely randomize design with four replications. Data were subjected to analysis of variance (ANOVA) and differences between treatment means were established with Dunnet T3 test. Total phenolics in soil (unsterile soil, sterile soil and unsterile soil plus charcoal) were separately regressed against root length of arrowleaf sida. The results are given in the text with $p < 0.05$ adopted as the criterion of significance. Statistical analysis was via SPSS 7.5 program.

3. Results

3.1. Effect of straw leachates on arrowleaf sida grown in unsterile soil and in unsterile soil mixed with activated charcoal

Unsterile soil treated with sugarcane straw leachate significantly inhibited root elongation of arrowleaf sida when compared with water control (Fig. 1). Maximum reduction on root length was 56.2 ± 2.1% at T1 and minimum significant reduction was 25.8 ± 1.6% at T3. Straw leachate did not significantly stimulate or inhibit shoot elongation (not shown).

No significant differences were observed on root elongation of arrowleaf sida grown in unsterile soil and unsterile soil mixed with activated charcoal, when they were treated with water (Fig. 1). Addition of activated charcoal eliminated root inhibition at T3 (Fig. 1). Roots of arrowleaf sida were significantly shorter in unsterile soil treated with T1 (29.1 ± 1.5%) and T2 (25.2 ± 1.8%) compared with those grown in unsterile soil mixed with charcoal and amended with the same leachate concentrations.

3.2. Residual effect of straw leachate in unsterile soil

Root inhibition in unsterile soil treated with T1 persisted when pregerminated seeds of arrowleaf sida were sown at 0 day (71.5 ± 1.3%) to 7 days (24.7 ± 1.8%) after leachate incorporation (Fig. 2a). Root inhibition disappeared when weed seeds were sown in soil at 10 days after leachate incorporation. Total phenolics in soil treated with T1 was 28.5 ± 2.5 μg g^{-1} at 0 day and decreased to 4.3 ± 1.2 μg g^{-1} at 10 days (Fig. 2b). In contrast, total phenolics was nearly constant in unsterile soil at 0, 1, 2, 3 and 7 days after water irrigation and was significantly lower than in unsterile soil at 0, 1, 2, 3 and 7 days after leachate incorporation. Linear regression analysis of root length and content of total phenolics in unsterile soil at different times after leachate incorporation indicated a negative correlation ($r^2 = 0.87$, $p < 0.05$).

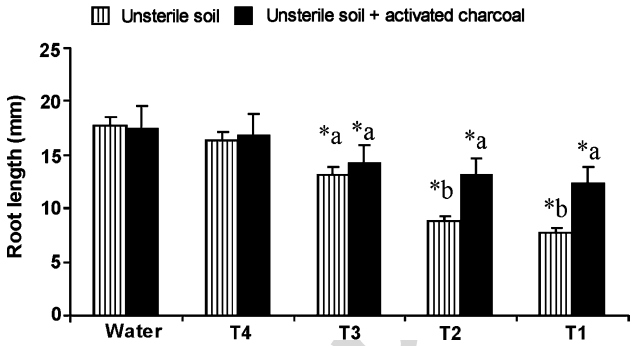

Fig. 1. Root elongation of arrowleaf sida in unsterile soil treated with sugarcane straw leachate (T1: 65 g dry straw L^{-1}; T2: 45 g dry straw L^{-1}; T3: 19 g dry straw L^{-1}; T4: 7 g L^{-1}) and unsterile soil plus activated charcoal treated with straw leachate (T1–T4). Soil treated with water and soil mixed with activated charcoal (4 mg g^{-1} of dry soil) treated with water served as controls, respectively. Asterisks (*) indicate significant differences from control ($p < 0.05$) and bar indicates standard deviation. Different letters indicate significant differences between root elongation in unsterile soil and in unsterile soil mixed with activated charcoal ($p < 0.05$) within a given straw leachate concentration.

3.3. Effect of straw leachates on arrowleaf sida grown in sterile and unsterile soils

Root elongation of arrowleaf sida was significantly inhibited in unsterile soil amended with T1 (52.7 ± 1.9%) and T2 (46.5 ± 1.6%). This inhibition was higher than that observed in sterile soil for T1 (32.5 ± 2.2%) and T2 (20.6 ± 1.1%) (Fig. 3).

3.4. Effect of leachate from straw ashes on growth of arrowleaf sida

Seedling elongation of arrowleaf sida was not significantly different in unsterile soil treated with leachate of straw ashes respect to water control (not shown).

3.5. Soil analysis

Levels of total phenolics were not significantly different in unsterile soil (4.1 ± 0.3 μg g^{-1}) or unsterile soil mixed with charcoal (4.1 ± 0.5 μg g^{-1}) and sterile soil (5.0 ± 0.5 μg g^{-1}) when they were treated with water (Fig. 4). Levels of total phenolics were significantly higher in unsterile soil treated with T2 (10.1 ± 0.2 μg g^{-1}) and T1 (11.0 ± 0.5 μg g^{-1}) than in unsterile soil mixed with activated charcoal treated with

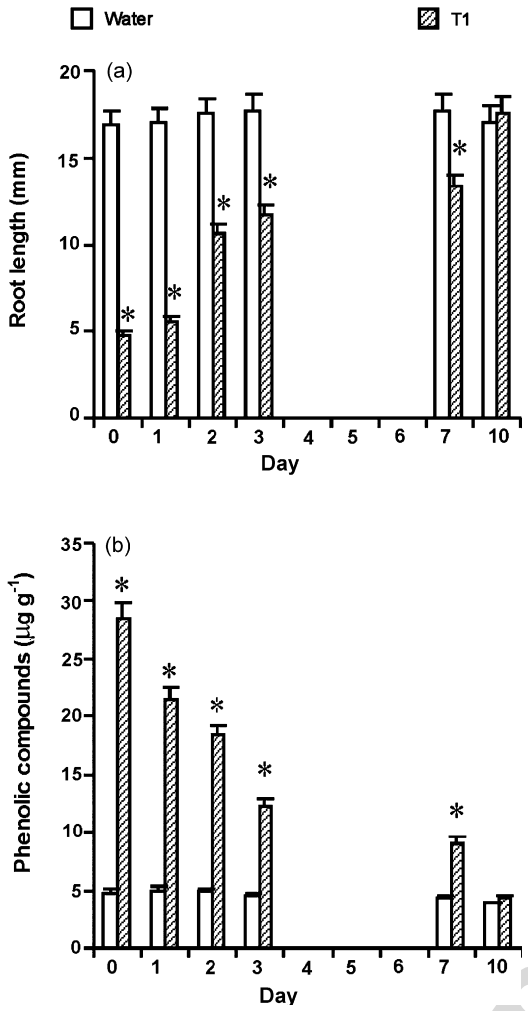

Fig. 2. Residual effect of a straw leachate (T1) incorporated to unsterile soil. T1 was incorporated at day 0. Pregerminated seeds of arrowleaf sida were sown at different dates (day 0, 1, 3, 7 and 10) after T1 incorporation. Root elongation was measured 7 days after date sowing (a). Soil total phenolics ($\mu g\, g^{-1}$ of soil) measured at each sowing date is also shown (b). Controls are shown for each sowing date and consisted in soil treated with water incorporated at day 0. Asterisks (*) indicate significant differences from corresponding control ($p < 0.05$) and bar indicates standard deviation.

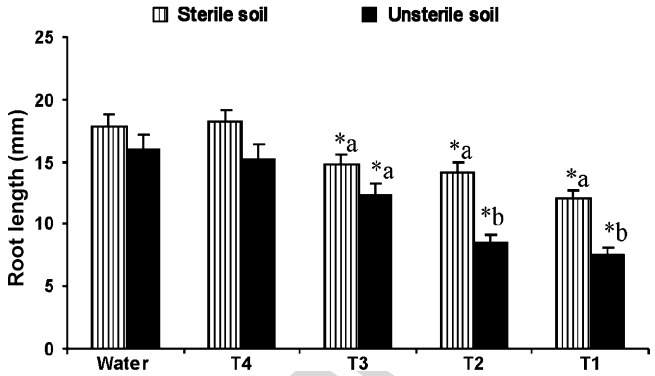

Fig. 3. Root elongation of arrowleaf sida when grown in unsterile and sterile soil treated with different amounts of sugarcane straw leachate (T1: 65 g dry straw L^{-1}; T2: 45 g dry straw L^{-1}; T3: 19 g dry straw L^{-1}; T4: 7 g L^{-1}). Unsterile and sterile soil treated with water served as controls, respectively. Asterisks (*) indicate significant differences from control ($p < 0.05$) and bar indicates standard deviation. Different letters indicate significant differences between root elongation in unsterile soil and in sterile soil ($p < 0.05$) within a given straw leachate concentration.

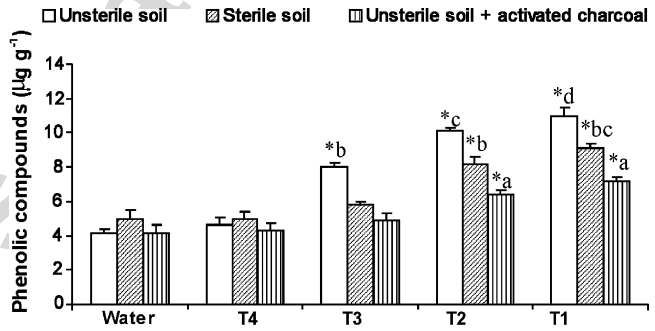

Fig. 4. Total phenolic ($\mu g\, g^{-1}$) levels in unsterile soil treated with sugarcane straw leachate (T1: 65 g dry straw L^{-1}; T2: 45 g dry straw L^{-1}; T3: 19 g dry straw L^{-1}; T4: 7 g L^{-1}), in sterile soil treated with sugarcane straw leachate (T1–T4) and in unsterile soil mixed with activated charcoal treated with sugarcane straw leachate (T1–T4). Unsterile soil, sterile soil and unsterile soil mixed with activated charcoal treated with water served as the respective controls. Values are shown as mean ± standard deviation. Asterisks (*) indicate significant differences from control ($p < 0.05$) and bar indicates standard deviation. Different letters indicate significant differences among total phenolic compounds in unsterile soil, in sterile soil and in unsterile soil mixed with activated charcoal ($p < 0.05$) within a given straw leachate concentration.

Table 1
Chemical characteristics of unsterile soil treated with leachates of straw ashes

	MO (%)	Soluble Salts (Electrical conductivity expressed in dS m^{-1})	pH	N total (%)	NO_3^- (ppm)	NH_4^+ (ppm)
Water	5.1 ± 0.1	0.41 ± 0.01	6.5 ± 0.1	0.19 ± 0.01	6.9 ± 0.5	44.5 ± 1.0
TS4	5.0 ± 0.1	0.49 ± 0.03	6.5 ± 0.1	0.19 ± 0.02	8.2 ± 1.0	44.4 ± 1.2
T4	5.1 ± 0.1	0.47 ± 0.05	6.5 ± 0.1	0.18 ± 0.01	7.6 ± 1.2	44.6 ± 1.0
TS3	5.1 ± 0.1	0.58 ± 0.02*	6.6 ± 0.2	0.19 ± 0.04	9.5 ± 1.1*	44.5 ± 0.9
T3	5.1 ± 0.1	0.48 ± 0.01	6.6 ± 0.2	0.19 ± 0.03	8.7 ± 1.0*	44.4 ± 1.2
TS2	5.1 ± 0.1	0.69 ± 0.01*	6.6 ± 0.2	0.19 ± 0.01	9.2 ± 1.3*	44.5 ± 0.9
T2	5.1 ± 0.1	0.58 ± 0.02*	6.6 ± 0.1	0.18 ± 0.02	10.4 ± 1.1*	44.7 ± 1.1
TS1	5.1 ± 0.1	0.72 ± 0.03*	6.6 ± 0.2	0.19 ± 0.01	10.7 ± 1.0*	40.1 ± 1.0
T1	5.1 ± 0.1	0.65 ± 0.05*	6.7 ± 0.1	0.19 ± 0.02	11.3 ± 1.2*	45.7 ± 1.2

TS4: 0.6 g of dry straw ashes L^{-1}, TS3: 2 g of dry straw ashes L^{-1}, TS2: 5 g of dry straw ashes L^{-1} and TS1: 7 g of dry straw ashes L^{-1}) and sugarcane straw leachates (T4: 7 g of dry straw L^{-1}, T3: 19 g of dry straw L^{-1}, T2: 45 g of dry straw L^{-1} and T1: 65 g of dry straw L^{-1}. Values are shown as mean ± standard deviation.

* Significantly different from the control ($p < 0.05$).

Table 2
Micro and macronutrients in unsterile soil treated with leachates of straw ashes (TS4, TS3, TS2 and TS1) and straw leachates (T4, T3, T2 and T1) of sugarcane

	Mg^{2+} (mg/100 g[a])	Ca^{2+} (mg/100 g[a])	Na^{+} (mg/100 g[a])	K^{+} (mg/100 g[a])	Mn^{2+} (ppm)	Zn^{2+} (ppm)	Fe^{3+} (ppm)	PO_4^{-} (ppm)
Water	26.5 ± 1.0	251.9 ± 2.3	8.9 ± 1.2	45.4 ± 1.5	394.0 ± 2.1	38.0 ± 1.4	17.0 ± 0.3	59.6 ± 1.0
TS4	25.1 ± 1.1	257.3 ± 2.8	8.6 ± 0.9	49.0 ± 0.9	357.3 ± 1.9	34.8 ± 1.6	17.4 ± 0.2	65.4 ± 0.9
T4	29.1 ± 1.4	240.4 ± 3.0	8.4 ± 1.1	51.3 ± 1.2	396.8 ± 2.0	32.3 ± 1.4	17.1 ± 0.1	64.4 ± 0.5
TS3	33.8 ± 1.5*	256.4 ± 2.1	7.9 ± 1.2	59.6 ± 0.4*	379.3 ± 1.9	27.5 ± 1.3	17.9 ± 0.3	69.4 ± 0.3*
T3	31.3 ± 2.5*	236.8 ± 2.5	8.6 ± 1.1	57.9 ± 0.8	389.2 ± 1.8	21.3 ± 1.7*	16.8 ± 0.2	65.6 ± 0.8
TS2	41.5 ± 1.6*	256.4 ± 2.1	8.7 ± 1.0	67.9 ± 0.9*	426.1 ± 1.7	23.8 ± 1.5*	17.3 ± 0.2	70.4 ± 0.5*
T2	36.9 ± 2.1*	222.4 ± 2.3	9.0 ± 1.1	55.6 ± 1.2*	390.0 ± 2.0	17.0 ± 1.1*	17.5 ± 0.1	69.4 ± 1.0*
TS1	44.0 ± 2.1*	248.1 ± 2.5	8.3 ± 1.1	75.5 ± 2.0*	418.1 ± 2.0	18.0 ± 1.4*	17.1 ± 0.2	69.3 ± 0.9*
T1	31.9 ± 1.7*	224.1 ± 2.4	8.0 ± 1.3	61.8 ± 1.5*	395.2 ± 1.8	22.2 ± 2.0*	17.2 ± 0.1	70.1 ± 1.1*

Values are shown as mean ± standard deviation. (T4: 7 g of dry straw L^{-1}, T3: 19 g of dry straw L^{-1}; T2: 45 g of dry straw L^{-1} and T1: 65 g of dry straw L^{-1}; TS4: 0,6 g of dry straw ashes L^{-1}; TS3: 2 g of dry straw ashes L^{-1}; TS2: 5 g of dry straw ashes L^{-1} and TS1: 7 g of dry straw ashes L^{-1}).
[a] grams of soil dry matter.
* Significantly different from the control ($p < 0.05$).

T2 ($6.4 \pm 0.3\ \mu g\ g^{-1}$) and T1 ($7.2 \pm 0.2\ \mu g\ g^{-1}$) or sterile soil treated with T2 ($8.2 \pm 0.4\ \mu g\ g^{-1}$) or T1 ($9.1 \pm 0.3\ \mu g\ g^{-1}$). No significant differences were observed in unsterile soil treated with leachate of sugarcane straw ashes respect to unsterile soil treated with water (not shown). Regression of total phenolics in soil (unsterile soil, sterile soil and unsterile soil mixed with charcoal, separately regressed) against root elongation was linear. An increase in soluble salts, Mg^{2+}, K^{+}, PO_4^{-} and NO_3^{-} and a decrease in Zn^{2+} was observed in unsterile soil treated with straw leachate and leachate of straw ashes (Tables 1 and 2). No significant differences were detected in pH, organic matter, ammonium and nitrate contents of unsterile soil after treatment with straw leachate and leachate of straw ashes (Table 2).

3.6. Malondialdehyde production, peroxidase activity and proline accumulation

Straw leachate incorporated to unsterile soil significantly increased proline content and MDA production of root tissues from arrowleaf sida proportionally with the increase of leachate concentration (Fig. 5). Proline content in the cotyledons also increased while no significant differences were observed in MDA cotyledon contents between arrowleaf sida grown in straw leachate and water (Fig. 6). Root GPX activity increased with straw leachate concentration to reach a maximum at T3 ($55.6 \pm 1.1\ \mu mol\ min^{-1}\ g\ fw^{-1}$) and then decreased (Fig. 5b) while cotyledon GPX activity was not significantly different between water and leachate treatments (not shown). Root proline content and MDA production were significantly higher in unsterile soil and unsterile soil mixed with activated charcoal treated with T1 and T2 respect to water controls and unsterile soil treated with leachate of straw ashes (Fig. 5a and c). When treated with straw leachate, roots grown in unsterile soil had a higher significant GPX activity than those grown in unsterile soil mixed with activated charcoal (Fig. 5b). No significant differences were observed in GPX activity between roots grown in unsterile soil treated with straw ashes and those grown in the water control (not shown).

4. Discussion

The primary effect of sugarcane straw leachate was a significant reduction in root elongation of arrowleaf sida. This finding agrees with previous results suggesting that water soluble constituents from sugarcane straw can reduce the competitive ability of sensitive weeds through an inhibition in root growth (Sampietro and Vattuone, 2006b). Several concentrations of straw leachate were assayed because preliminary observations indicated field variations in straw density. These changes in density are also common in crops under no-tillage system (Politycka and Lipinska, 2005). As can be seen in Fig. 1, field variations in sugarcane straw densities could be an important factor that regulates the ocurrence or absence of straw phytotoxicity (Liebl and Worsham, 1983; Sampietro and Vattuone, 2006b).

Unsterile soil amended with leachate of straw ashes was not inhibitory to arrowleaf sida suggesting that straw inorganic constituents were not responsible of the observed phytotoxicity (Hamdi et al., 2001). Unsterile soil was mixed with activated charcoal and then treated with straw leachate to confirm the involvement of organic compounds on root growth inhibition (Inderjit and Foy, 1999). Activated charcoal is a wide range adsorbant with little affinity for inorganic electrolytes (Cheremisinoff and Ellerbusch, 1978) and is frequently used to separate the direct inhibitory effect of organic compounds from indirect mechanisms of growth interference (Wardle and Nilsson, 1997; Sampietro and Vattuone, 2006a). After addition of activated charcoal, the straw leachate was less inhibitory to root growth suggesting that organic soil molecules available subsequent to straw leachate incorporation had a direct inhibitory effect on root growth and were adsorbed by activated charcoal.

The inhibitory activity of sugarcane straw leachate disappeared 10 days after incorporation to unsterile soil. This could be due to microbial degradation, chemical decomposition, and (or) sorption of straw leachate phytotoxins (Inderjit and Weiner, 2001). These soil processes would be responsible of the observed decrease of total phenolics over the time in unsterile soil (Dalton, 1999; Sampietro and Vattuone, 2006a).

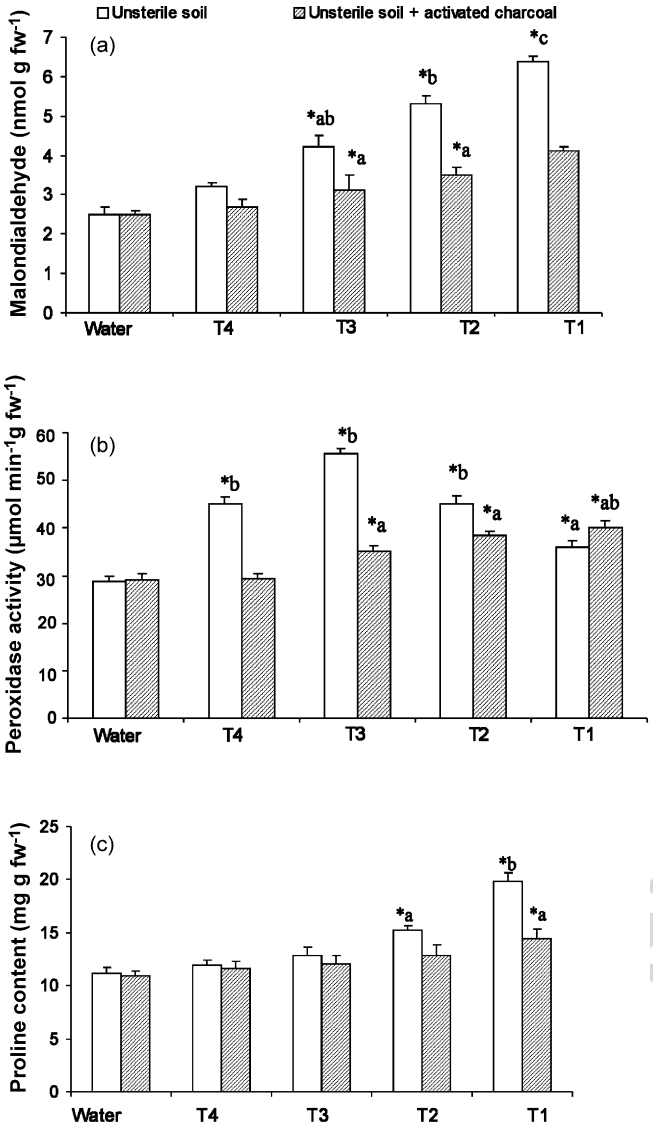

Fig. 5. Malondialdehyde production (a), peroxidase activity (b), and proline content (c) of arrowleaf sida roots grown in unsterile soil treated with sugarcane straw leachate (T4–T1), and in unsterile soil mixed with activated charcoal treated with sugarcane straw leachate (T4–T1). Data are shown as mean ± standard deviation. Asterisks (*) indicate significant differences from control ($p < 0.05$) and bar indicates standard deviation. Different letters indicate significant differences in malondialdehyde production, peroxidase activity or proline content between arrowleaf sida roots grown in unsterile soil and in unsterile mixed with activated charcoal ($p < 0.05$) within a given straw leachate concentration.

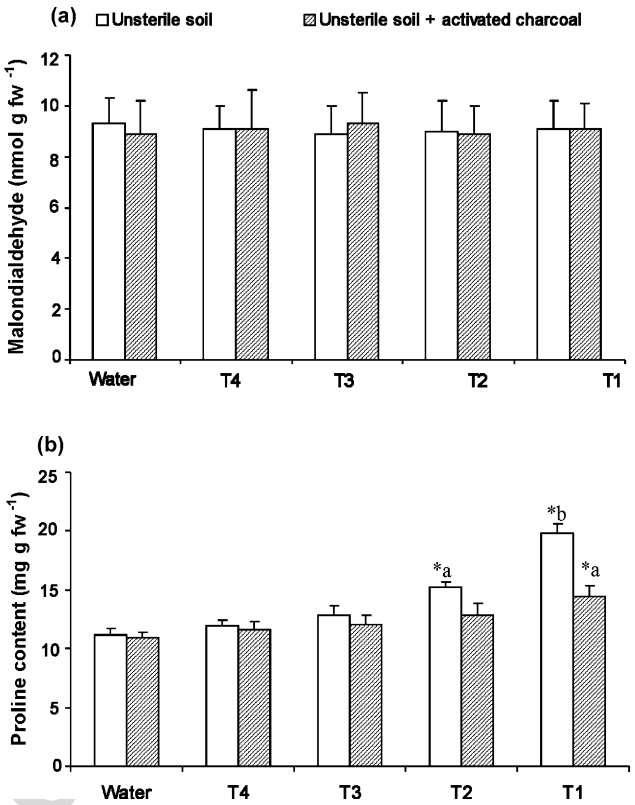

Fig. 6. Malondialdehyde production (a), and proline content (b) of arrowleaf sida cotyledons grown in unsterile soil treated with sugarcane straw leachate (T4–T1), and in unsterile soil plus activated charcoal treated with sugarcane straw leachate (T4–T1). Data are shown as mean ± standard deviation. Asterisks (*) indicate significant differences from control ($p < 0.05$) and bar indicates standard deviation. Different letters indicate significant differences in malondialdehyde production or proline content between arrowleaf sida cotyledons grown in unsterile soil and in unsterile plus activated charcoal ($p < 0.05$) within a given straw leachate concentration.

In general, a significant increase in soil phenolics content was observed after incorporation of sugarcane straw leachate. The decline in root elongation of arrowleaf sida was strongly correlated with the increase of phenolic contents in soil (unsterile, sterile and unsterile mixed with charcoal) suggesting that phenolics could participate in the observed growth inhibition. The content of soluble salts and some ions was also determined in unsterile soil treated with straw leachate and leachate of straw ashes because both organic and inorganic soil components can be important in determining the phytotoxicity of soil treated with a plant debris (Inderjit and Dakshini, 1999; Sampietro and Vattuone, 2006a). Leachates from straw ashes can change soil properties, which can be related with their effect (inhibition, stimulation or no effect) on growth of receptor plants (Hamdi et al., 2001; Inderjit et al., 2004). If leachates from straw ashes do not inhibit plant growth, soil modifications associated with them should not explain growth suppression on the receptor plants. Content of some ions in unsterile soil treated with straw leachate or ashes of sugarcane straw was higher than that determined in soil treated with water. Modifications of the measured soil properties were very similar after incorporation of the leachates. Hence, the evaluated soil characteristics suggest that straw phytotoxicity on arrowleaf sida is the consequence of a direct action of soil organic components present after addition of straw leachate rather than a direct inhibitory activity derived from the incorporation of straw inorganic constituents or an indirect inhibition related with modifications in other soil characteristics.

Unsterile soil treated with T1, T2 and T3 was more inhibitory on root growth of the assayed weed than sterile soil suggesting that microorganisms increased the inhibitory activity of the straw leachate (Inderjit et al., 2004; Sampietro and Vattuone,

2006a). Microbial immobilization of soil inorganic constituents is often proposed to explain this response (Harper, 1977). In our experiments it cannot be argued because both straw leachate and leachate of straw ashes increased the soil content of most of the determined inorganic ions, including NO_3^-. Considering levels of phenolics in unsterile and sterile soil treated with straw leachate, it is likely that microbial activity may have influenced the quantitative and (or) qualitative availability of phenolics, which in turn could have a significant influence on root growth of arrowleaf sida (Inderjit et al., 2004).

Root MDA production of arrowleaf sida grown in unsterile soil increased with increasing levels of the straw leachate. Guaiacol peroxidase activity, however, decreased at concentrations higher than T3 suggesting that root seedlings were under an oxidative stress. The increment of proline content in roots grown in unsterile soil was proportional to the increase of MDA production suggesting that proline could act in this situation as a scavenger of free radicals to prevent oxidative damage in root tissues (Smirnoff and Cumbes, 1989). In contrast, proline accumulation in the cotyledons was not accompanied with evidences of oxidative stress. Oxidative damage in arrowleaf sida roots could contributes to water imbalance of the whole plant as a consequence of the loss of root cell membrane integrity (Sampietro et al., 2006; Zeng et al., 2001). Proline accumulation in the cotyledons could be necessary for osmotic adjustment arised from a water stress (Lutts et al., 1996). Fig. 5 indicates that GPX activity, proline content and lipid peroxidation were significantly lower in root seedlings grown in unsterile soil mixed with charcoal than in those grown in unsterile soil alone suggesting that organic constituents from sugarcane straw induced the observed proline accumulation and the oxidative stress. The possible participation of phenolics in this process should not be rule out since several phenolics are able to induce oxidative stress and changes in plant water status (Baziramakenga et al., 1995; Sampietro et al., 2006).

5. Conclusion

Our results indicate that soil treated with sugarcane straw leachate interferes with root growth of arrowleaf sida. The provided evidence suggests that organic molecules from straw leachate or their microbial transformation products in unsterile soil had a direct action on seedling growth inducing root oxidative stress and foliar and root proline accumulation. Phenolics from the straw leachate or their microbial transformation products could participate in sugarcane straw interference through a direct influence on seedling growth of arrowleaf sida, although interaction and/or participation of other organic compounds from the straw leachate in the interference process could not be excluded.

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