

Phytotoxic Catechin Leached by Seeds of the Tropical Weed *Sesbania virgata*

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Abstract *Sesbania virgata* (Cav.) Pers (wand riverhemp) is a fast-growing tropical legume species that has been used for revegetation of riparian forests and rehabilitation of degraded areas and that exhibits an invasive behavior in certain regions of Brazil. Preliminary studies have shown that seed leachates inhibit the germination and development of seedlings of some crop species. In this study, we report that the seed leachates of *S. virgata* inhibit the growth of *Arabidopsis thaliana* and rice. The flavonoid (+)-catechin is found in high amounts in these leachates. It was active at concentrations of 50 $\mu\text{g ml}^{-1}$, and its effect was not distinguishable from the (+)-catechin obtained from a commercial source. We found that (+)-catechin is located in the seed coat and is rapidly released in high concentrations (235 μg per seed) at the beginning of imbibition. Quercetin was also detected in the seed coat of *S. virgata*, but it was not released from the seeds. Other phytotoxic compounds in the seed leachates were also detected. The fact that *S. virgata* releases high amounts of (+)-catechin,

which also has antimicrobial activity, and other phytotoxins from its seeds at the earliest stages of its development might represent some adaptative advantage to the seedling that contributes to its invasive behavior and successful establishment in different soils.

Keywords *Sesbania virgata* · Seed leachate · (+)-Catechin · Allelopathy · Allelochemicals · Quercetin

Introduction

The seed stage is a crucial phase of plant development that allows plants to effectively establish in the natural environment. One strategy that enables the success of the early establishment of plants is the release of secondary metabolites and other low-molecular-weight compounds at germination, which can inhibit the proliferation of soil pathogens and the germination and growth of competing plants (Nelson 2004).

Some studies have examined how phytotoxic secondary metabolites exuded by the roots can influence the ability of a plant to become invasive in a new environment, such as the enantiomers (\pm)-catechin exuded by *Centaurea maculosa* Lam. (Bais et al. 2002) and lactones released by *Echinochloa crusgalli* (L.) Beauv. (Xuan et al. 2006). Although these studies have examined only those compounds that are exuded through the plant root tissues, seeds are also able to rapidly release allelochemicals after the beginning of the imbibition process that contribute to the invasive behavior of some species (Ndakidemi and Dakora 2003 and references therein).

The tropical legume *Sesbania virgata* (Cav.) Pers (Syn. *S. marginata* Benth) is a perennial, fast-growing shrub native to South America. It is 2–4 m in height and occurs in

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Central, Southeast, and South Brazil, Argentina, Uruguay, and Paraguay. It produces a lot of seeds with long-term viability that are dispersed within indehiscent legume fruits that float in the water (Pott and Pott 1994). It has been described as invasive in flood and damp soil, especially in inundated rice plantations (Kissmann and Groth 1999). This species has been used for revegetation of riparian forests, soil erosion control, and rehabilitation of degraded areas (Pott and Pott 1994).

Previous studies have demonstrated that *S. virgata* seed leachates can affect the germination and growth of the radicle of some crop species (lettuce, tomato, and radish; Simões and Braga, unpublished data). It also has been reported that the seeds of various other *Sesbania* species contain toxic compounds that are able to restrict the growth of other plant species (Buta 1983; Powell et al. 1990; Van Staden and Grobbelaar 1995). *Sesbania* species generally present abundant seed production and rapid growth, occurring naturally near rivers, in flood places, and in modified soils. *S. punicea* (Cav) Benth. is described as a noxious weed in South Africa, invading abandoned fields and out-competing natural vegetation, and forming a dense population. This behavior seems to be associated with the presence of the potent alkaloid sesbanimide in the seeds that can inhibit the seedling growth of several species (Gorst-Allman et al. 1984; Van Staden and Grobbelaar 1995). As reported by Singh et al. (2007), under field conditions, intercropping of *Sesbania* species with cultivated plants reduces the density of grass weeds. Due to its invasive behavior and demonstrated ability to release phytotoxins from its seeds, *S. virgata* may fit the seed-related allelopathic invasiveness pattern of this genus; however, the chemicals responsible for the phytotoxicity have not yet been identified.

Despite the fact that seed leachates of *S. virgata* contain large amounts of sugars released during the mobilization of the storage cell wall carbohydrates by the developing embryo (Buckeridge and Dietrich 1996), no microbial contamination has been observed during the germination process. This lack of a microbial presence suggests the release of antimicrobial compounds during the early stages of germination. As the phytotoxic compounds produced are unknown, the aim of the present work was to isolate and identify phytotoxins present in the *S. virgata* seed leachates. We tested compounds on *Arabidopsis thaliana* and rice under laboratory conditions. We report that (+)-catechin is the major compound released by *S. virgata* seeds.

Methods and Materials

Seed Germination Seeds of *S. virgata* were collected from natural populations in the region of São Paulo, Brazil. Six

thousand seeds were selected, scarified, and placed on two Whatman 1 filter papers in 15-cm-diameter Petri dishes along with 0.7 ml of distilled water per seed (50 seeds per dish). The plates were incubated for 3 days at 25°C with a photoperiod of 12 h.

Extraction and Isolation During the third day of germination, seeds were removed from the Petri dishes, the leachates were collected, and the filter papers were washed $\times 3$ with 105 ml of distilled water (representing three times the initial water volume added to each dish). The leachates of each dish were combined, filtered through a *nylon* membrane, freeze-dried, and weighed (6 g). The resultant powder was dissolved in 300 ml distilled water and extracted sequentially with the same volume of hexane and ethyl acetate ($\times 3$ each). The aqueous extract was freeze-dried and re-dissolved in methanol, and evaporated under a vacuum. The ethyl acetate extracts were chromatographed in a regular Merck silica gel 60 (0.063–0.200 mm) column (80 \times 3 cm) and eluted with a gradient of dichloromethane (DCM) and MeOH (0 \rightarrow 100% MeOH). Fifty fractions were collected and assembled according to their thin-layer chromatography (TLC) profiles to yield ten fractions (A–J). One of the largest phytotoxic fractions collected (fraction H, 110 mg) was re-fractioned in a flash chromatography system with a silica gel column (1 \times 20 cm) and DCM/MeOH (9:1) as an isocratic solvent system.

Seeds removed from the Petri dishes were also freeze-dried, triturated with a Walita mixer, extracted $\times 3$ with pure ethanol (0.7 ml per seed), evaporated, and partitioned with hexane, ethyl acetate, and methanol as described above. The ethyl acetate fraction (600 mg) was also submitted to a regular Merck silica gel 60 (0.063–0.200 mm) column (2 \times 20 cm) with a gradient with DCM and MeOH (0 \rightarrow 100% MeOH). The 60 fractions collected were compiled into 15 fractions according to their TLC profiles, and one fraction (six) that contained pure quercetin was detected.

Circular Dichroism Spectroscopy and ^1H NMR Analyses Quercetin and catechin isolated from *S. virgata* seeds and leachates were identified by ^1H NMR spectra analyses in CD_3OD and compared to standards purchased from Sigma by using a Varian INOVA 400-MHz Fourier spectrometer. Catechin was also analyzed by its circular dichroism (CD) spectrum in methanol on an Aviv model 202 spectrometer and compared with the standard. (+)-Catechin showed a cotton effect at 280 nm ($\Delta\epsilon = -1.511$; Korver and Wilkins 1971).

Quantification of Isolated Compounds Seeds of *S. virgata* were germinated as described above, and every day, between the beginning of the imbibition and the final day

of germination (1–6 days), a set of 40 seeds was removed from the Petri dishes. Tissues were separated (seed coat, endosperm, and embryo), freeze-dried, and extracted $\times 3$ with ethanol (0.7 ml per seed). Ethanol was evaporated, and the extracts were suspended in 1 ml distilled water and partitioned with 1 ml ethyl acetate ($\times 4$). After drying, extracts were solubilized in MeOH (high-performance liquid chromatography, HPLC, grade) and injected into a C18 Dionex Acclaim column (150 \times 4.6 mm) in an HPLC mass spectrometry (HPLC-MS) consisting of a system P680 pump, an ASI-100 autosampler, and a UVD170U UV detector coupled to a Thermo Finnigan Surveyor MSQ mass spectral detector. The samples were eluted with acetic acid (0.1%) in water (eluent A) and 0.1% acetic acid in methanol (eluent B) in a linear gradient from 10% of B (3 min) to 90% of B (90 min). Compounds were quantified by using Sigma standards for catechin and quercetin chromatographed under the same conditions. Leachates obtained from each germination day were also collected and extracted with ethyl acetate and analyzed by HPLC-MS as described above.

Phytotoxicity Bioassays Seeds of *A. thaliana* (L.) Heynh. ecotype Columbia (Col-0) purchased from Lehle Seed Co. (Texas, USA) were surface-sterilized with 20% commercial sodium hypochlorite (0.4% active chloride) for 2 min, washed $\times 4$ in sterile distilled water, and further germinated on solid Murashige and Skoog (1962) basal medium in Petri dishes at 25 \pm 2 $^{\circ}$ C and a 16:8 h L/D photoperiod. After 5 days, the *A. thaliana* seedlings were relocated to sterile 12-well plates (one plant per well) that contained 1 ml of liquid MS basal media. They were placed on an orbital shaker under the growth conditions described above to oxygenate the roots properly. Six replicates were used per treatment. After 1 day, catechin and other fractions obtained from *S. virgata* seed leachates and a commercial standard of (+)-catechin (Sigma) were applied to the liquid MS containing the *A. thaliana* seedlings. The substances were dissolved in methanol or ethanol, and 10 μ l of the extracts containing different concentrations were applied to the wells. Controls were performed using only the solvents. After 7 days, the length and biomass of the plant root tissues were evaluated.

Seeds of inundated rice (*Oryza sativa* L.) cultivar IAC 106 (Instituto Agronômico de Campinas, SP, Brazil) were washed rapidly with 80% ethanol and then surface sterilized with 20% commercial sodium hypochlorite (0.4% active chloride) for 20 min. After that, seeds were washed three times with sterile distilled water then immersed in a 0.2% fungicide solution (Derosal Bayer) for 30 min and subsequently washed three times with sterile distilled water. Rice seeds were germinated in 250-ml flasks

that contained solid MS basal medium at 25 \pm 2 $^{\circ}$ C with 16:8 h L/D photoperiod. After 6 days, seedlings were transferred to tubes (2.3 \times 15 cm) that contained 2 ml of liquid MS basal medium (one seedling per tube). The bioassay was performed as described for *A. thaliana*.

Statistical Analyses All statistical analyses were performed by using Winstat software (Microsoft, USA). Growth parameters were subjected to analysis of variance (ANOVA), and significant differences between means were determined by using *LSD* test at 1% probability.

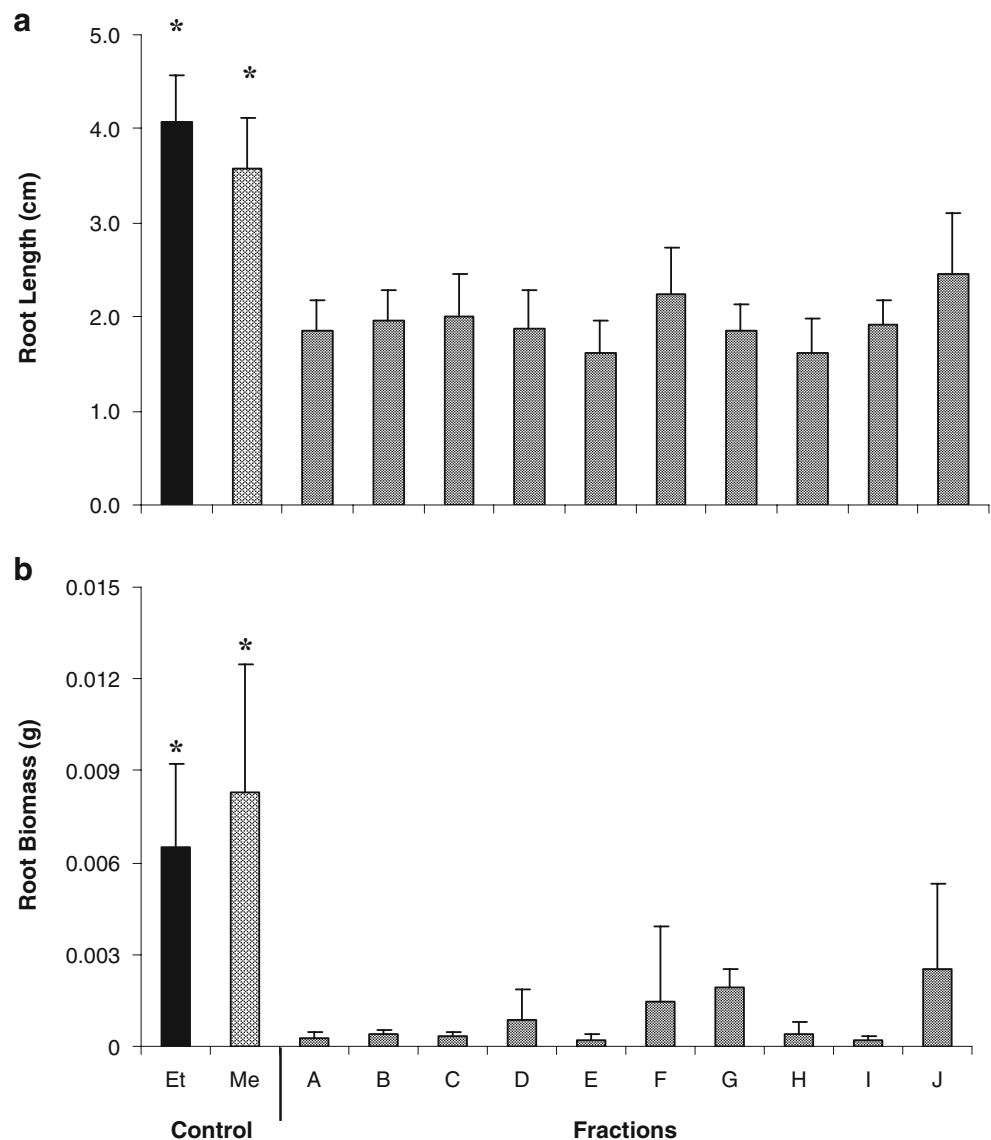
Results

Fractions A–J obtained from the ethyl acetate extract of the seed leachates caused significant reduction in root length and root biomass in *A. thaliana* plants grown in MS media when compared to the controls (Fig. 1). The ^1H NMR spectrum from one of the largest fractions collected (H) and also from the G fraction was identical to that of the standard bioflavonoid catechin. When fraction H was rechromatographed, two of the 20 fractions were pure catechin (F2 and F3, containing 23 and 17 mg, respectively). ^1H NMR of these fractions confirmed that the isolate compound from *S. virgata* seed leachates was catechin, and the CD spectrum analyses revealed that the molecule was the enantiomer (+)-catechin (data not shown).

Different concentrations of (+)-catechin isolated from *S. virgata* seed leachates were tested against *A. thaliana* seedlings, and activity was compared with commercial (+)-catechin. Statistical analyses (*LSD*, ANOVA, $P=0.01$) showed that there were no differences between the treatments with catechin from Sigma and from *S. virgata* (Fig. 2). The effect of the concentrations of 50 and 100 $\mu\text{g ml}^{-1}$ on root length was not different from the effect of methanol alone on control plants (Fig. 2a); however, differences were detected in root biomass of plants among these treatments (Fig. 2b). (+)-Catechin appeared to be phytotoxic to root tissues of *A. thaliana* at the concentration of 50 $\mu\text{g ml}^{-1}$. The concentrations of 200 and 500 $\mu\text{g ml}^{-1}$ were toxic, causing a darkening of the plant roots, a total inhibition of lateral root formation (not shown), and drastic reduction in root biomass (Fig. 2).

Toxicity of (+)-catechin purified from *S. virgata* seed leachates was also observed on rice, and this level of toxicity was similar to that found with catechin purchased from Sigma. With both, toxicity was detected only in the response of root biomass (Fig. 3). This effect was statistically different from control plants when a concentration of 100 $\mu\text{g ml}^{-1}$ of catechin or higher was used (Fig. 3b). Root damage, however, was observed even with the lowest concentration (50 $\mu\text{g ml}^{-1}$).

Fig. 1 Root length (a) and root biomass (b) of *A. thaliana* seedlings grown for 7 days in liquid MS media containing one of the fractions A–J collected from the *S. virgata* seed leachates' chromatography. Each fraction was administered at a concentration of $200 \mu\text{g ml}^{-1}$ of MS media. Control plants were treated with the same volume of ethanol (Et) used to solubilize fractions A–D or methanol (Me) used to solubilize fractions E–J. The asterisk indicates a significant difference between the means of the treatments and the controls determined by the test LSD (ANOVA, $P=0.01$). Bars represent the SD of the means ($N=6$)



The flavonoid quercetin was isolated from the seed extracts (25 mg), but it was not exuded by *S. virgata* seeds (Fig. 4). It was phytotoxic to *A. thaliana* (data not shown). Both (+)-catechin and quercetin are located in the seed coat tissues of *S. virgata*; these compounds were not detected by HPLC-MS analyses in the extracts of the embryo or endosperm tissues (data not shown).

During the germination of *S. virgata*, we found that (+)-catechin was rapidly released from the seed coat tissues on the first day of imbibition at levels up to $235 \mu\text{g}$ per seed (Fig. 4). After this first release, levels in the seed leachates decreased from the second day of imbibition. Based on the data of Fig. 4, the amount of (+)-catechin released from one seed was estimated as higher than $400 \mu\text{g}$ until the sixth day after the beginning of imbibition. The quantity of catechin and quercetin in the seed coat also decreased during germination, but the amount of (+)-catechin in the

seed coat was considerably lower than that measured in the seed leachates (Fig. 4).

Discussion

High concentrations of constitutive phenolic compounds in the seed coat have been reported in *Sesbania* species. Some are rapidly released during imbibition and are found in the soil surrounding germinating seeds (Ceballos et al. 1998). We found that (+)-catechin was rapidly released from seed coat tissues of *S. virgata* on the first day of imbibition at much higher (approximately ten times) levels than those reported for other species of *Sesbania* (Ceballos et al. 1998) and *Lespedeza* (Buta and Lusby 1986).

Catechin has shown phytotoxic effects on a variety of plants, but the level of phytotoxicity varies depending

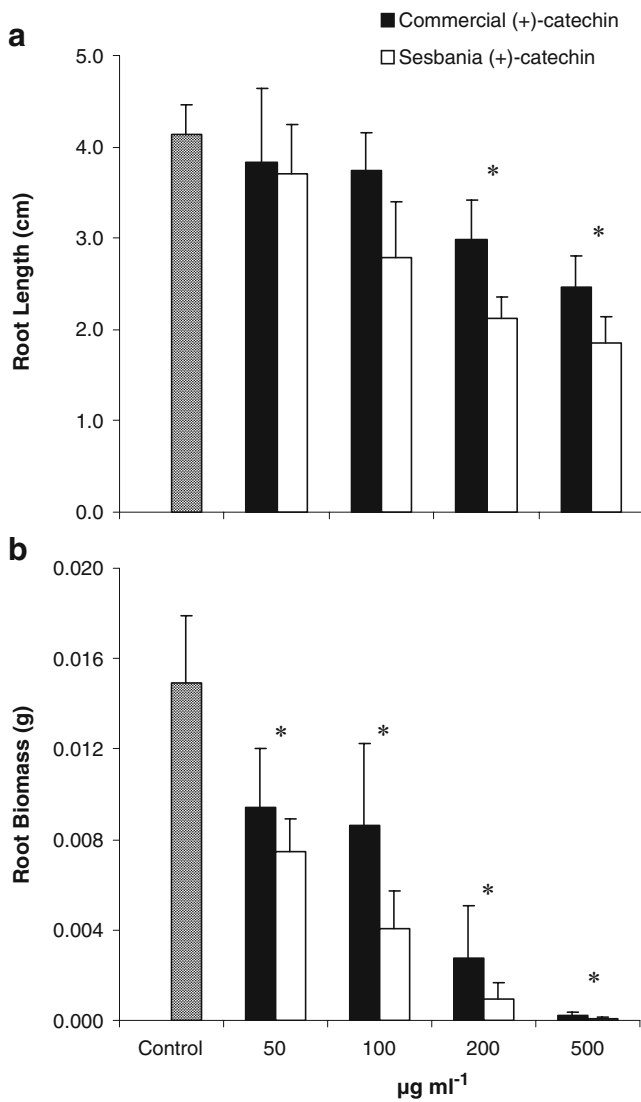


Fig. 2 Effect of different concentrations of (+)-catechin derived from *S. virgata* seed leachates and commercial (+)-catechin on the length (a) and root biomass (b) of *A. thaliana* seedlings after 7 days of treatment. Control plants were treated only with methanol. The asterisk indicates a significant difference between the means of the treatments and the control determined by the test LSD (ANOVA, $P=0.01$). Bars show the SD of the means ($N=6$)

on the species tested and the experimental conditions (Buta and Lusby 1986; Bais et al. 2002, 2003; Iqbal et al. 2003; Perry et al. 2005a; Thelen et al. 2005; D’Abrosca et al. 2006; Thorpe 2006; Weir et al. 2006; Furubayashi et al. 2007). In addition, (+)-catechin has antimicrobial activity and inhibits the growth of the pathogenic soil bacteria *Erwinia carotovora*, *E. amylovora*, *Xanthomonas campestris*, and *Pseudomonas fluorescens* (Veluri et al. 2004). (±)-Catechin also acts as an autoregulator of seed germination and seedling development in *Centaurea maculosa* and *Lespedeza* sp. (Buta and Lusby 1986; Perry et al. 2005b). Seed germination in *S. virgata* seems not to

be affected by its coat leachates, but additional experiments are needed to definitively determine this.

The inhibitory effect of (+)-catechin isolated from seed leachates of *S. virgata* on *A. thaliana* growth was similar to that observed by using commercial catechin. Treated plants showed a reduction in root biomass (Fig. 2b) when compared to control plants. This was most likely due to the characteristic effect of the bioflavonoid catechin in inhibiting the development of lateral roots of seedlings of *A. thaliana*. The phytotoxicity of catechin on root cell tissues of *A. thaliana* has been previously described as caused by condensation of the cytoplasm generated by the rapid induction of reactive oxygen species, followed by a subsequent increase of Ca^{2+} and acidification of the cyto-

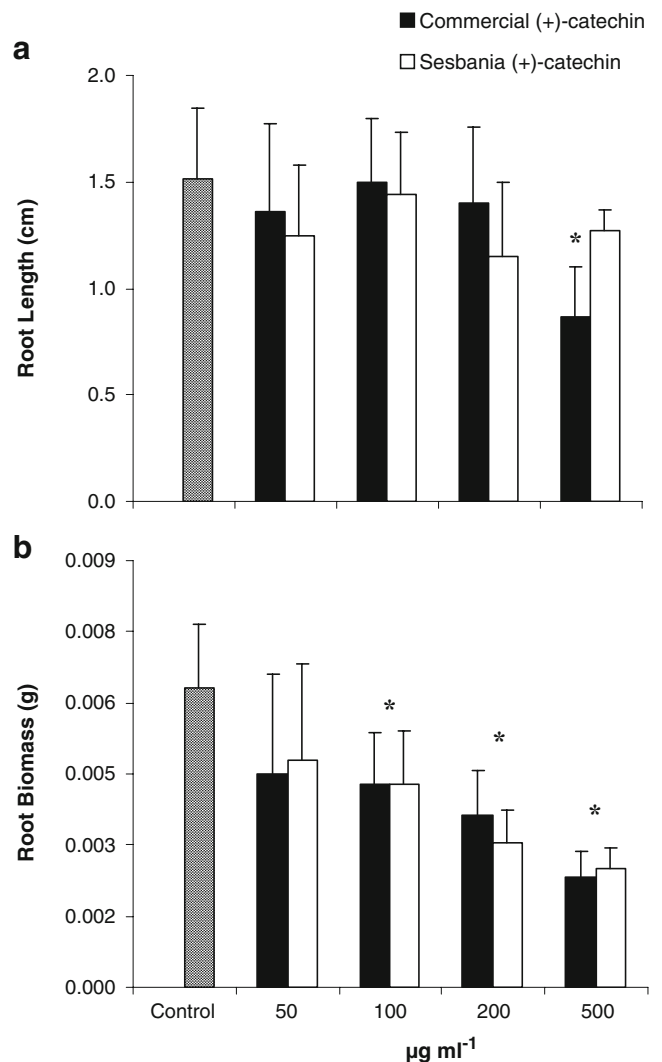
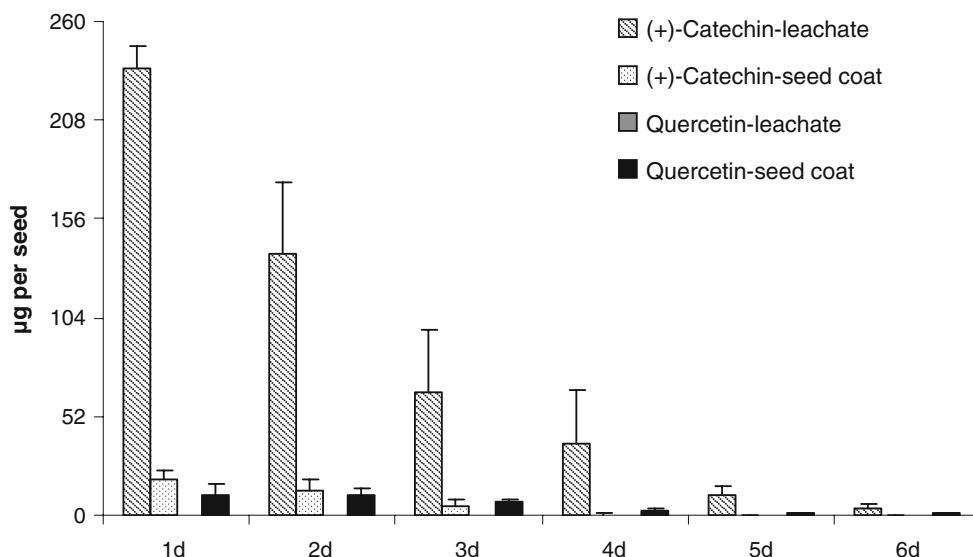


Fig. 3 Effect of different concentrations of (+)-catechin derived from *S. virgata* seed leachates and commercial (+)-catechin on root length (a) and root biomass (b) of rice seedlings after 7 days of treatment. Control plants were treated only with methanol. The asterisk indicates a significant difference between the means of the treatments and the control determined by the test LSD (ANOVA, $P=0.01$). Bars show the SD of the means ($N=6$)

Fig. 4 Quantification of quercetin and catechin present in the seed coat and in the seed leachates of *S. virgata* during the beginning of imbibition (1 day—first day) to the ending of the germination process (6 days—sixth day). Analyses were performed by HPLC-MS, and compounds were quantified and detected by using a commercial standard. Bars represent the SD of the means ($N=3$)



plasm, which induces cell death (Bais et al. 2003). We also found phytotoxic effects of seed leachate catechin on rice seedlings (Fig. 3).

S. virgata is considered an invasive species in flood and damp soils, especially inundated rice plantations (Kissmann and Groth 1999). Therefore, the presence of this toxin in seed leachates may indicate an allelopathic strategy during germination and may contribute to the protection of *S. virgata* seeds against the attack of potential pathogens.

S. virgata is a pioneer species that produces many long-term viable seeds within indehiscent legume fruits. These fall on the ground and are further dispersed by water and/or wind, as reported for other *Sesbania* species (Pott and Pott 1994; Ceballos et al. 1998). The species thus forms a transitory seedbank, and its allelochemicals are released during seed imbibition. As described by Ceballos et al. (1998), the anatomical organization of *Sesbania* seeds is finely adapted to facilitate the rapid deployment of chemicals present in the seed coat, contributing to the rapid mobilization of these compounds toward the zone around the imbibed seed. Although these chemicals can be altered by reactions with other substances in the soil, microbial breakdown, and compound stability, the presence of active substances in the seed coat and their early release in high amounts at the beginning of imbibition suggest that they may play an ecological role. An early pulse of high catechin concentrations could contribute to enabling the species to rapidly and temporarily control the growth of nearby plants under specific environmental conditions. Early allelopathic effects may be advantageous to the competitive outcome; and resource depletion depends on the size of competing plants (Lattera and Bazzalo 1999). Moreover, antimicrobial activity of (+)-catechin could provide a hostile environment to potential microbial invaders.

Root-secreted (\pm)-catechin may contribute to the invasive behavior of the noxious weed *Centaurea maculosa*, which shows a devastating effect on species native to the Northeast of USA (Bais et al. 2002). Despite recent findings that indicate that the amounts of catechin found in soils around *C. maculosa* are lower than those previously reported to be phytotoxic (Blair et al. 2005, 2006), probably due to the low stability of the catechin in the soil, a short-term pulse of high amounts could be sufficient to confer some advantage at critical periods of development. This has been observed recently in the field for root exudates of *C. maculosa* (Perry et al. 2007). It is also important to note that the dynamics of root exudation is different from the release of substances from seed coats. As reported by Phillips et al. (2006), plants are able to recover part of their root-exuded compounds, sometimes with an influx rate that exceeds their efflux. *S. virgata* produces seeds with the ability to control water imbibition in early stages of germination, and as reported for other species of *Sesbania*, the anatomical structure of the seed coat plays a role in the establishment of an allelochemical-rich zone during imbibition (Ceballos et al. 1998). Additionally, seed coats remain in the environment, possibly increasing the amounts of soil catechin. The compound continues to be released up to 6 days after the beginning of imbibition as shown in Fig. 4.

The detection of other phytotoxic fractions derived from the *S. virgata* seed leachates that do not contain (+)-catechin in their molecular composition (Fig. 1) suggests that this seed can likely release other prospective phytotoxins that may relate to the invasive character and to adaptive mechanisms. Our data indicate that the seed coat of *S. virgata* contains the bioflavonoids (+)-catechin and quercetin, but that only (+)-catechin seems to operate as an allelochemical. Additional experiments are needed to

determine the presence of other active compounds and also the possibility of co-interactions that could potentiate phytotoxic effects.

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References

- BAIS, H. P., WALKER, T. S., STERMITZ, F. R., HUFBAUER, R. A., and VIVANCO, J. M. 2002. Enantiomeric-dependent phytotoxic and antimicrobial activity of (+)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol.* 128:1173–1179.
- BAIS, H. P., VEPACHEDU, R., GILROY, S., CALLAWAY, R. M., and VIVANCO, J. M. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301:1377–1380.
- BLAIR, A. C., HANSON, B. D., BRUNK, G. R., MARRS, R. A., WESTRA, P., NISSEN, S. J., and HUFBAUER, R. A. 2005. New techniques and finds in the study of a candidate allelochemical implicated in invasion success. *Ecol. Lett.* 8:1039–1047.
- BLAIR, A. C., NISSEN, S. J., BRUNK, G. R., and HUFBAUER, R. A. 2006. A lack of evidence for an ecological role of the putative allelochemical (\pm)-catechin in spotted knapweed invasion success. *J. Chem. Ecol.* 32:2327–2331.
- BUCKERIDGE, M. S., and DIETRICH, S. M. C. 1996. Mobilisation of the raffinose family oligosaccharides and galactomannan in germinating seeds of *Sesbania marginata* Benth. (Leguminosae-Faboideae). *Plant Sci.* 117:33–43.
- BUTA, G. J. 1983. Linoleic acid as a plant growth inhibitor from seeds of *Sesbania punicea*. *J. Nat. Prod.* 46:775.
- BUTA, J. G., and LUSBY, W. R. 1986. Catechins as germination and growth inhibitors in *Lespedeza* seeds. *Phytochemistry* 25: 93–95.
- CEBALLOS, L., HOSSAERT-MCKEY, M., MCKEY, D., and ANDARY, C. 1998. Rapid deployment of allelochemicals in exudates of germinating seeds of *Sesbania* (Fabaceae): roles of seed anatomy and histolocalization of polyphenolic compounds in anti-pathogen defense of seedlings. *Chemoecology* 8:141–151.
- D'AMBROSCA, B., DELLAGRECA, M., FIORENTION, A., ISIDORI, M., MONACO, P., and PACIFICO, S. 2006. Chemical constituents of the aquatic plant *Schoenoplectus lacustris*: evaluation of phytotoxic effects on the green alga *Selenastrum capricornutum*. *J. Chem. Ecol.* 32:81–96.
- FURUBAYASHI, A., HIRADATE, S., and FUJII, Y. 2007. Role of catechol structure in the adsorption and transformation reactions of L-Dopa in soils. *J. Chem. Ecol.* 33:239–250.
- GORST-ALLMAN, C. P., STEYN, P. S., VLEGGAAR, R., and GROBBELAAR, N. 1984. Structure elucidation of sesbanimide using high-field NMR spectroscopy. *J. Chem. Soc.* 1:1311–1314.
- IQBAL, Z., HIRADATE, S., NODA, A., ISOJIMA, S., and FUJII, Y. 2003. Allelopathic activity of buckwheat: isolation and characterization of phenolics. *Weed Sci.* 51:657–662.
- KISSMANN, K. G., and GROTH, D. 1999. Plantas Infestantes e nocivas. BASF, São Bernardo do Campo.
- KORVER, O., and WILKINS, C. K. 1971. Circular dichroism spectra of flavonols. *Tetrahedron* 27:5459–5465.
- LATERA, P., and BAZZALO, M. E. 1999. Seed-to-seed allelopathic effects between two invaders of burned Pampa grasslands. *Weed Res.* 39:297–308.
- MURASHIGE, T., and SKOOG, F. 1962. A revised medium for rapid growth and bioassay with tissue culture. *Physiol. Plant.* 15:473–497.
- NDAKIDEMI, P. A., and DAKORA, F. D. 2003. Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. *Funct. Plant Biol.* 30:729–745.
- NELSON, E. B. 2004. Microbial dynamics and interactions in the sphere. *Annu. Rev. Phytopathol.* 42:271–309.
- PERRY, L. G., JOHNSON, C., ALFORD, E. R., VIVANCO, J. M., and PASCHKE, M. W. 2005a. Screening of grassland plants for restoration after spotted knapweed invasion. *Restor. Ecol.* 13:725–735.
- PERRY, L. G., THELEN, G. C., RIDENOUR, W. M., WEIR, T. L., CALLAWAY, R. M., PASCHKE, M. W., and VIVANCO, J. M. 2005b. Dual role for an allelochemical: (\pm)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *J. Ecol.* 93:1126–1135.
- PERRY, L. G., THELEN, G. C., RIDENOUR, W. M., CALLAWAY, R. M., ASCHKE, M. W., and VIVANCO, J. M. 2007. Concentrations of the allelochemical (\pm)-catechin in *Centaurea maculosa* soils. *J. Chem. Ecol.* 33:2337–2344.
- PHILLIPS, D. A., FOX, T. C., and SIX, J. 2006. Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO₂. *Global Change Biol.* 12:561–567.
- POTT, A., and POTT, V. J. 1994. Plantas do Pantanal. EMPRAPA/CPAP/SPI, Corumbá.
- POWELL, R. G., PLATTNER, R. D., and SUFFNESS, M. 1990. Occurrence of sesbanimide in seeds of toxic *Sesbania* species. *Weed Sci.* 38:148–152.
- SINGH, S., LADHA, J. K., GUPTA, R. K., BHUSHAN, L., RAO, A. N., SIVAPRASAD, S., and SINGH, P. P. 2007. Evaluation of mulching, intercropping with *Sesbania* and herbicide use for weed management in dry-seeded rice (*Oryza sativa* L.). *Crop Protect* 26:518–524.
- THELEN, G. C., VIVANCO, J. M., NEWINGHAM, B., GOOD, W., BAIS, H. P., LANDRES, P., CAESAR, A., and CALLAWAY, R. M. 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecol. Lett.* 8:209–217.
- THORPE, A. 2006. Biochemical effects of *Centaurea maculosa* on soil nutrient cycles and plant communities. PhD dissertation, University of Montana, Missoula.
- VAN STADEN, J., and GROBBELAAR, N. 1995. The effect of sesbanimide and *Sesbania* seed extracts on germination and seedling growth of a number of plant species. *Environ. Exp. Bot.* 35:321–329.
- VELURI, R., WEIR, T. L., BAIS, H. P., STERMITZ, F. R., and VIVANCO, J. M. 2004. Phytotoxic and antimicrobial activities of catechin derivatives. *J. Agric. Food Chem.* 52:1077–1082.
- WEIR, T. L., BAIS, H. P., STULL, V. J., CALLAWAY, R. M., THELEN, G. C., RIDENOUR, W. M., BHAMIDI, S., STERMITZ, F. R., and VIVANCO, J. M. 2006. Oxalate contributes to the resistance of *Gaillardia grandiflora* and *Lupinus sericeus* to a phytotoxin produced by *Centaurea maculosa*. *Planta* 223:785–795.
- XUAN, T. D., CHUNG, M. III, KHANH, T. D., and TAWATA, S. 2006. Identification of phytotoxic substances from early growth of barnyard grass (*Echinochloa crusgalli*) root exudates. *J. Chem. Ecol.* 32:895–906.