Acta Physiologiae Plantarum (2022) 44: 96
https://doi.org/10.1007/s11738-022-03431-5

ORIGINAL ARTICLE

Seed leachates of the tropical legume *Sesbania virgata*: their effects on germination and seedling growth of tomato and rice

Leilyane Conceição de Souza Coelho[1,2] · Daiane Salete Broch Mignoni[3,4] · Claudio José Barbedo[5] · Marcia Regina Braga[6]

Received: 20 July 2021 / Revised: 20 July 2021 / Accepted: 18 August 2022 / Published online: 28 August 2022

Abstract

Allelopathic substances from seed leachates can mediate plant interspecific relationships. (+)-Catechin is the main phytotoxin leached by the seeds of *Sesbania virgata* with effects on native and crop species. In this article, we hypothesised that *S. virgata* seed leachates affect mobilization of seed storage carbohydrates in crop species. We used tomato and rice as target species, which store mannan and starch as seed polysaccharides, respectively. Experiments of seed-to-seed co-germination with *S. virgata* and germination of tomato and rice with of *S. virgata* seed leachates or commercial catechin showed that *S. virgata* affected tomato germination and the root and shoot growth in both species. Commercial catechin only reduced seedling growth. The reducing sugar content decreased in both rice and tomato and the total sugar content in rice only when seeds germinated with *S. virgata* seed leachates. The endo-β-mannanase activity was inhibited in tomato seeds germinated with *S. virgata* seed leachates but not with commercial catechin. These results suggest that although catechin interferes with germination and seedling growth of agronomic species, the inhibition of storage carbohydrate mobilization by *S. virgata* seems to be related to other allelochemicals present in the seed leachates rather than to catechin.

Keywords Allelochemicals · Catechin · Germination · Seed storage carbohydrates

Introduction

Sesbania virgata Cav. Pers (Fabaceae) is a Fabaceae species that occurs mainly in Brazilian gallery forests. This pioneer shrub produces large amounts of seeds that remain viable for long periods, forming transient banks in the soil (Pott and Pott 1994; Potomati and Buckeridge 2002; De Araújo et al. 2004). *Sesbania virgata* tolerates high heavy metal concentrations contributing to their phytostabilization in contaminated soils (Branzini et al. 2012) and behaves as an invasive species in irrigated crops such as rice (Kissmann and Groth 1999; Lorenzi 2000). In Brazil, the species has invaded areas of caatinga and other associated biomes, mainly riparian forests, becoming dominant and preventing the regeneration of native species (Andrade 2006; Souza et al. 2011).

Invasive plants are characterised by their ability to produce substances that play a major role in plant–plant interactions, strongly influencing the dynamics of agroecosystems, and guaranteeing their establishment in different habitats. Allelopathic substances or phytotoxins can exert mostly negative effects on the associated plants, influencing growth and crop productivity (Zahedi and Ansari 2011). Seeds of

Communicated by P. Wojtaszek.

✉ Daiane Salete Broch Mignoni
daianeb.mignoni@gmail.com

[1] Postgraduate Program in Cellular and Structural Biology, Campinas State University Zeferino Vaz, Barão Geraldo, Campinas, SP 13083-970, Brazil

[2] Present Address: Laboratory of Agricultural Cultures and Caatinga in the Submédio São Francisco – LACACSSF, University of Pernambuco-UPE, Rodovia BR 203, Km 2 s/n - Vila Eduardo, Petrolina, PE 56328-900, Brazil

[3] Postgraduate Program in Plant Biodiversity and Environment, Institute of Botany, Av. Miguel Estéfano 3687, Água Funda, São Paulo, SP 04301-902, Brazil

[4] Center for Natural Sciences and Humanities, Federal University of ABC, R. Arcturus 03, Jardim Antares, São Bernardo Do Campo, SP 09606-070, Brazil

[5] Seed Department, Institute of Botany, Av. Miguel Estéfano 3687, Água Funda, São Paulo, SP 04301-902, Brazil

[6] Physiology and Biochemistry Department, Institute of Botany, Av. Miguel Estéfano 3687, Água Funda, São Paulo, SP 04301-902, Brazil

S. virgata release allelochemicals that can contribute to its invasive behaviour. Simões et al. (2008) have shown that seed leachates of *S. virgata* negatively impacted the germination and growth of tomato, rice, lettuce, and *Arabidopsis thaliana*. Allelochemicals of *S. virgata* also affected native co-occurring species in their natural environment (Veronesi 2013) but to a lesser extent than agronomic species (El Id et al. 2015). The flavonoid (+)-catechin is the major compound leached by *S. virgata* seeds that inhibits plant root growth (Simões et al. 2008). Catechin mediates the successful invasion of new environments by *Centaurea stoebe* (He et al. 2009; Thorpe et al. 2009), but whether this allelochemical exerts reasonably phytotoxic in the soil to affect other plant species is still controversial (Blair et al. 2005; 2006; Chobot et al. 2009; Wang et al. 2013).

Besides catechin, the seed coat of quiescent seeds and the leachates of *S. virgata* have high concentrations of abscisic acid (ABA) (Tonini et al. 2006; Mignoni et al. 2017), which interferes in the activity of hydrolysing enzymes during seed galactomannan mobilization (Potomati and Buckeridge 2002; Tonini et al. 2006, 2010). Simões et al. (2008) described the presence of quercetin in the seed coat of *S. virgata*, which was not detected in the seed leachates.

Seed leachates of *S. virgata* inhibited growth and carbohydrate mobilization of the non-native species *Leucaena leucocephala.* In contrast, catechin and ABA solely did not show inhibitory effects on this alien species under laboratory conditions (Mignoni et al. 2017).

Phytotoxins are found in different parts of the plant, such as leaves, roots, fruits, bark, trunks, and seeds (Souza Filho et al. 2011 and refs therein). Alkaloids, flavonoids, benzoxazinoids (hydroxamic acids, benzoxazolinones, and hydroxamic acid methylated derivatives) and glycoside resins have been described as allelopathic substances, which might affect germination, prevent microbial action, contribute to the invasive behaviour of some plants or avoid the noxious influence of other species (Ndakidemi and Dakora 2003; Souza Filho et al. 2011). Allelopathic studies have been carried out using mainly aqueous or organic plant extracts (Zhang et al. 2021). However, few studies (Souza Filho 2002; Villagrasa et al. 2006; Souza Filho et al. 2011) have described the effects of phytotoxins released by seeds. Studies carried out under both laboratory and field conditions demonstrated that seeds and aqueous leachates of *Vigna mungo*, a legume used in crop rotation during winter and spring regimes in India, affected the seed germination and root growth of rice, lentil, and corn (Suman et al. 2002).

Environmental stresses strongly impact seed germination, which is a critical stage of plant development (Luo et al. 2018). Carbohydrates, lipids, and proteins are the main seed reserve compounds, which sustain the embryo development until the seedling formation (Weidlich et al. 2010). Few studies describe the effect of phytotoxins released by seeds of tropical native species on the growth and carbohydrate degradation of other plant species (Simões et al. 2008; Zhang et al. 2011; El Id et al. 2015; Ma et al. 2015; Mignoni et al. 2018). Germination rates and root growth of native grasses declined by the effect of the water-soluble seed leachate from *Liguraria virgaurea* (Zhang et al. 2011). The phytochemicals produced by *S. virgata* seeds had a strong inhibitory effect on germination and seedling growth and also produced a delay in galactomannan degradation and raffinose family oligosaccharides mobilisation on *L. leucocephala* (Mignoni et al. 2018). In contrast, the effects of *S. virgata* seeds were less intense on the initial growth of native species, with co-occurring species, than on agronomic species (El Id et al. 2015). Thus, in natural areas, *S. virgata* seeds can restrict the growth of alien species, reduce some alien competitors, and influence interspecific competition with co-occurring species (Simões et al. 2008; El Id et al. 2015; Mignoni et al. 2018).

In our study, we hypothesized that seed leachates of the tropical legume *S. virgata* would exert their inhibitory effect on the growth of seedlings of agronomic species by affecting mobilisation of storage carbohydrates. As a model to test our hypothesis, we selected two crop species, tomato and rice, which accumulate mannans (structural carbohydrates) and starch (a non-structural carbohydrate), respectively, as seed storage carbohydrates.

Material and methods

Material

Individuals of *Sesbania virgata* (Cav.) Pers. growing in ten natural tree populations in Lavras, MG, Brazil (45°00′25′′W 21°13′35′′S, 45°00′24′′W 21°13′30′′S, 45°00′39′′ 21°13′11′′S) were used as a seed source. Tomato seeds (*Solanum lycopersicum* L. cv. Santa Cruz Kada) Isla® were obtained in the local market, and EMBRAPA *"Arroz e Feijão",* Santo Antônio de Goiás, GO (Brazil) kindly donated rice seeds (*Oryza sativa* L. cv. Ourominas).

Co-germination assays

After scarification with sandpaper, seeds of *S. virgata* were disinfested by immersion in a 10% sodium hypochlorite solution (containing 2.5% active chloride w/v) for 10 min and washed four times with autoclaved distilled water. In the first co-germination assay, seeds were placed on a piece of filter paper moistened with 5 mL of distilled water inside transparent plastic boxes (11 × 11 × 3 cm). The seed proportion was 1:5 for *S. virgata*: tomato and 1:3 for *S. virgata*: rice. Incubation was performed in a germination chamber (BOD model 347 FG, Fanem®) at 25 °C under a 12-h

photoperiod for 5 days. For the control, the seeds of both agronomic species were germinated in the absence of *S. virgata* seeds. The treatments were arranged in a completely randomised design with three replicates. Germination was recorded daily, and radicle protrusion was used as the germinative criteria. Seeds were considered germinated when root length achieved 2 mm. Root and shoot lengths were measured, and the germination speed index was calculated (GSI) (Maguire 1962).

Seed leachates

A thousand 5 mm-long seeds of *S. virgata* were scarified using sanding paper (P80 3 M). After disinfection by immersion in a 10% aqueous solution of commercial sodium hypochlorite (2% active chlorine v/v) for 20 min and four washes with distilled water, seeds were placed into 150 mm glass Petri dishes (50 seeds/dish) containing filter paper (Qualy®) moistened with 35 mL of sterile distilled water. Seeds were incubated for 48 h on growth chambers as described above. This incubation period was selected because it was previously reported as the period of greatest leakage of catechin from *S. virgata* seeds (Simões et al. 2008). Seed leachates were collected, freeze-dried, and weighed. Aqueous solutions (w/v) of crude seed leachates were prepared at 1, 2 or 4 mg mL^{-1} for further use. To estimate the concentration of catechin in the seed leachates, condensed tannins were quantified in the seed exudates by the vanillin method (Broadhurst and Jones 1978), using (+)—Catechin (Sigma Aldrich) as standard.

Germination assay with leachates

Tomato seeds were surface disinfested by immersion in 10% sodium hypochlorite (2% active chlorine v/v) for 30 min and washed four times with autoclaved distilled water. In the first experiment, 10 seeds were germinated on filter paper imbibed in 3 mL of aqueous seed leachates of *S. virgata* or 1, 2 or 4 mg mL^{-1} of (+)—catechin (Sigma Aldrich) for 5 days inside of Petri dishes (5 cm). Based on the results of this first experiment, 1 mg mL^{-1} of seed leachates was selected for further use. In a second experiment, 50 tomato seeds were germinated in Petri dishes (15 cm), on filter paper imbibed in 15 mL of aqueous seed leachates of *S. virgata* or 1 mg mL^{-1} of commercial (+)—catechin for 4 days. Control seeds were germinated on filter paper imbibed in autoclaved distilled water. The experiments were performed with three replicates. In both experiments, seeds were incubated in a growth chamber as described above, and seed germination, radicle, and shoot lengths, and fresh and dry masses of the seedlings were recorded.

Rice seeds were first disinfested with 80% ethanol (30 s), washed four times with autoclaved distilled water and sequentially immersed in a 10% aqueous solution of commercial hypochlorite sodium (2% active chlorine v/v) for 20 min. Seeds were washed with autoclaved distilled water, immersed in 0.2% aqueous solution of the fungicide Derosal® (Bayer) for 30 min, and washed four times with distilled water. Two experiments were carried out with the number of rice seeds and the volume of the solutions adjusted according to the size of the Petri dishes. In 5 cm-diameter Petri dishes, five rice seeds were germinated on paper filter containing 2 mL of seed leachates of *S. virgata* or 1, 2, and 4 mg mL^{-1} of (+)—catechin (Sigma-Aldrich) for 5 days. Based on the results of this first experiment, 1 mg mL^{-1} of seed leachates was selected for further use. In a second experiment, 20 rice seeds were germinated in 9 cm-diameter Petri dishes containing filter paper imbibed in 8 mL of leachates or 1 mg mL^{-1} of commercial (+)—catechin for 4 days. As a control, seeds were germinated on filter paper imbibed in autoclaved distilled water. The treatments were arranged in a completely randomised design with three replicates. The incubation period and parameters measured were the same described above.

Soluble sugar and starch analyses

The analyses were performed on seeds germinated for 4 days in the presence of 1 mg mL^{-1} of *S. virgata* leachates and 1 mg mL^{-1} of catechin. A hundred mg of fresh tomato or rice seeds of control, co-germinated seeds, and catechin treatments were subjected to the extraction of soluble sugars using 80% ethanol, in a water bath at 85 °C for three times of 15 min, according to Carvalho et al. (2013). Thereafter, the extracts were centrifuged for 10 min (2000 rpm, Sorvall® Super T21 centrifuge) and the supernatants pooled and concentrated in a rotary evaporator and resuspended in distilled water (2 mL). Quantification of total soluble and reducing sugars was performed colourimetrically according to the protocols described by Somogyi (1945) and Dubois et al. (1956). Glucose (Sigma Aldrich) was used as standard. Aliquots of these extracts were subjected to deionization in 50 × 8 cationic (100–200 mesh) and 1 × 8 anionic (52–100 157 mesh) Dowex columns (Mello et al. 2010). Soluble sugars were analysed (isocratic elution with 100 mM of sodium hydroxide (NaOH), 0.25 mL min^{-1} flow, 35 min) by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) using a Carbo-Pac PA1 column in a Dionex system (ICS-3000, USA). Mono and oligosaccharides were identified by comparison with commercial standards of glucose, fructose, sucrose, raffinose, stachyose, and verbascose (Sigma Aldrich) (Leduc et al. 2012).

Aliquots of the residues (10 mg) from the soluble sugar extraction were used for starch analysis, as described by Amaral et al. (2007) and modified according to Caccere et al.

(2013). Briefly, the residues were incubated with 0.5 mL of thermostable α-amylase (120 U mL^{-1}) of *Bacillus licheniformis* (EC 3.3.1.1, Megazyme), diluted in 10 mM MOPS buffer (3-morpholinopropane-1-sulfonic acid $C_7H_{15}NO_4S$) pH 6.5 and incubated at 75 °C for 30 min. This procedure was repeated twice, totalling 120 units of the enzyme. Samples were cooled to 50 °C (in bathing) and 0.5 ml of amyloglucosidase (30 U mL^{-1}) of *Aspergillus niger* (EC 3.2.1.3, Megazyme) in 100 mM sodium acetate ($C_2H_3NaO_2$) buffer pH 4.5 was added, followed by incubation at 50 °C for 30 min. This process was repeated twice, totalling 30 units of the enzyme. The enzymatic reaction was stopped by adding 100 µL 0.8 M of perchloric acid. The quantification of the enzymatic hydrolysis products was carried out after incubation with glucose PAP Liquiform reagent (Centerlab), containing the enzymes glucose oxidase and peroxidase (GOD-POD), 4-aminoantipyrine and phenol (pH 7.5) for 15 min at 30 °C. The absorbance was read at 490 nm using an ELISA microplate reader. Commercial glucose (Sigma Aldrich) was used as standard.

Enzymatic activity

The analyses were performed on 4-day-old seedlings developed in the presence of 1 mg mL^{-1} of *S. virgata* leachates and 1 mg mL^{-1} of catechin. The extraction of α-galactosidase (EC 3.2.1.22) using 50 mg of tomato and rice seeds germinated with *S. virgata* leachates, 1 mg mL^{-1} of commercial (+) – catechin or in the case of the control, with autoclaved distilled water (Reid and Meier 1973; Buckeridge and Dietrich 1996). The fresh tissues were pulverised using liquid nitrogen, homogenised in 1 mL of 20 mM Tris–HCl buffer pH 7.8, and maintained in an ice bath for 30 min. The extracts were centrifuged at 13,000 rpm for 5 min in an Eppendorf 5415R refrigerated microcentrifuge (5 °C). The enzymatic activity was evaluated in the supernatant by incubating 10 µL of the extract with 10 µL McIlvaine buffer (200 mM sodium phosphate + 100 mM citric acid) pH 4.4 and 10 µL of the specific substrate *p*-nitrophenyl-α-D-galactopyranoside (PNP, Sigma), at 45 °C for 20 min. After stopping the reaction with 1 mL of 200 mM sodium carbonate, the absorbance was read at 405 nm in a Shimadzu spectrophotometer (UV-1201). The amount of *p*-nitrophenol released (µmol *p*-nitrophenol min^{-1} DW mg^{-1}) was calculated using the molar extinction coefficient of *p*-nitrophenyl (18,400 M^{-1} cm^{-1}) (Reid and Meier 1973). Three replicates of all analyses were performed for each biological extract.

Fifty mg (fresh mass) of tomato and rice seeds was powdered in liquid nitrogen and used for α-amylase extraction (EC 3.2.1.1). The material was extracted with 1 mL of 50 mM potassium phosphate buffer pH 6.8 at 4 °C for 30 min with casual stirring. Afterwards, the supernatant was collected by centrifugation at 3000 *g* for 5 min. All the procedure was carried at 4 °C (Bernfeld 1955; Lara-Núñez et al. 2009).

The α-amylase activity was assessed by adding 50 µL of the enzyme extract to 50 µL of 1% starch solution in 50 mM potassium phosphate buffer pH 6.8, followed by incubation at 37 °C for 30 min. The reaction was interrupted by adding 50 µL of 3,5-dinitrosalicylic acid reagent (DNS) and boiling for 10 min. The quantification was performed by colourimetric assay at 570 nm in an ELISA microplate, using maltose (Sigma) as standard (Bernfeld 1955; Lara-Núñez et al. 2009). Pre-boiled extracts were considered as the zero-reaction time. Technical triplicates of all analyses were performed for each of the three biological replicates.

For the assay endo-β-1,4-mannanase (EC 3.2.1.78) activity, a hundred mg of tomato and rice seeds was germinated with seed leachates and 1 mg mL^{-1} of commercial (+)—catechin. In the case of the control, seeds were germinated with autoclaved distilled water. The extraction was performed with 1 mL of 50 mM sodium acetate buffer (pH 4.4) containing 0.02% sodium azide in an ice bath under stirring (vortex) for 15 min. After centrifugation (13,000 g, 4 °C, 5 min), aliquots of the supernatant (500 µL) were incubated with commercial β-Mannazyme tablets (Megazyme, lot 50,201) at 40 °C for 5 min. Exactly 10 min after the addition of the tablet, the reaction was stopped with 2% Trizma base (pH 8.5), and the incubation mixture maintained at room temperature for 5 min. The mixture was agitated and filtered through Whatman Nº 41 filter paper, and the absorbance was measured at 590 nm. Analyses were performed in triplicates, and an enzyme unit (U) was defined according to the beta-Mannazyme protocol (Megazyme, Lot 50,201).

A hundred mg of seedlings of each species was weighed and homogenised with 1 mL of 200 mM sodium phosphate buffer pH 7.5 containing 20% glycerol and 20 mM manganese sulphate (Shimon-Kerner et al. 2000). After centrifugation at 13000 *g* for 10 min in a refrigerated centrifuge, the supernatant was used for the analysis of the vacuolar and cytosolic invertase activity. The insoluble material was washed with 50 mM of HEPES sodium hydroxide buffer containing 0.5 mM of Na_2EDTA, 2.5 mM of ditioteitrol (DTT), 0.5% bovine serum albumin (BSA), and 1% polyvinylpyrrolidone (PVP), pH 7.5. After centrifugation, the residue was recovered and extracted with the same buffer containing 1 M of sodium chloride and incubated for 2 h in an ice bath. Thereafter, the extract was again centrifuged, and the supernatant analysed for cell wall (apoplastic) invertase activity.

The vacuolar activity was determined by adding 200µL of the enzymatic extract to 200 µL of 500 mM sodium acetate buffer containing 60 mM of sucrose and 0.01% of BSA pH 5.7. The mixture was incubated in a water bath at 40 °C for 30 min. The reaction was stopped by boiling for 5 min. Pre-boiled extracts were considered as the zero-incubation time.

Cytosolic neutral invertase activity was measured under the same conditions described above but at pH 7.5. For the acid cell wall invertase activity, the same incubation buffer was adjusted to pH 4.7. After boiling, incubation mixtures were centrifuged for 10 min.

The three invertase enzymes were analysed using the reagent Liquiform Glucose PAP (Centerlab®) that contains the enzymes glucose oxidase and peroxidase (GOD-POD) and 4-aminoantipyrine and phenol pH 7.5. The absorbance was read at 490 nm, using glucose (Sigma Aldrich) as standard. Analyses were performed in triplicates.

Protein assay

Protein was quantified in the extracts as described by Bradford (1976). Bovine serum albumin (BSA) (Sigma) was used in the standard curve. Analyses were performed in triplicates.

Experimental design and statistical analyses

All experiments followed a completely randomised design, with at least three replicates per treatment. The effects of co-germination with *S. virgata* seeds and enzymatic activities on target species were previously analysed with ANOVA, and the differences between means were compared with Tukey's test ($P \leq 0.05$). A two-way ANOVA was used for testing the effect of *S. virgata* sugar on the content of tomato and rice seeds. Significant differences between treatments were identified using the Bonferroni post hoc test. Statistical analyses were performed with GraphPad Prism 5.01.

A completely randomised design with three replications arranged in a factorial scheme (2 treatments × 3 concentrations) was used for testing seedling growth, fresh, and dry masses (Figs. 1 and 2).

Results

Influence of co-germination with *S. virgata* seeds

The general aspect of tomato and rice seedlings after the co-germination with *S. virgata* for 5 days is shown in figure S1 of the Supplementary material. Tomato seeds were severely affected by the co-germination with *S. virgata*, showing a 50% decrease in germination, and a decrease of approximately 70, 95 and 60% in the germination speed index, seedling initial growth and fresh mass, respectively (Table 1). In contrast, the co-germination of rice with *S. virgata* only showed a decrease in rice seedling growth. Reduction in rice shoot and root lengths was 76 and 86%, respectively, compared to the control (Table 1).

Effects of *S. virgata* leachates and catechin on target species

As shown in Table 2, we observed an inhibitory effect of the aqueous seed leachates of *S. virgata* on tomato germination at all concentrations assayed. Differences between doses above 1 mg mL^{-1} were found at days 3–5, with the strongest effect detected with 2 mg mL^{-1}. In contrast, catechin did not affect tomato germination at all concentrations tested

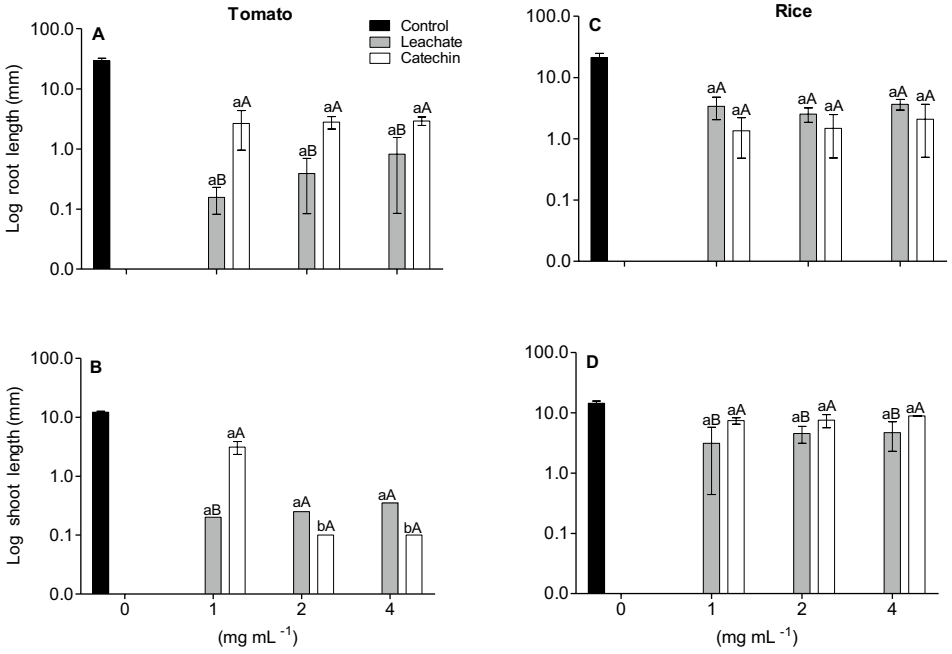

Fig. 1 Effect of different concentrations of seed leachates of *Sesbania virgata* and commercial catechin on the root and shoot lengths of tomato (**A**–**B**) and rice (**C**–**D**) seedlings germinated after four days of imbibition. The bars indicate the standard error ($N = 3$). Lower case letters compare concentrations within each treatment and upper case letters between treatments. Values with the same letters are not significantly different by Tukey's test ($P < 0.05$)

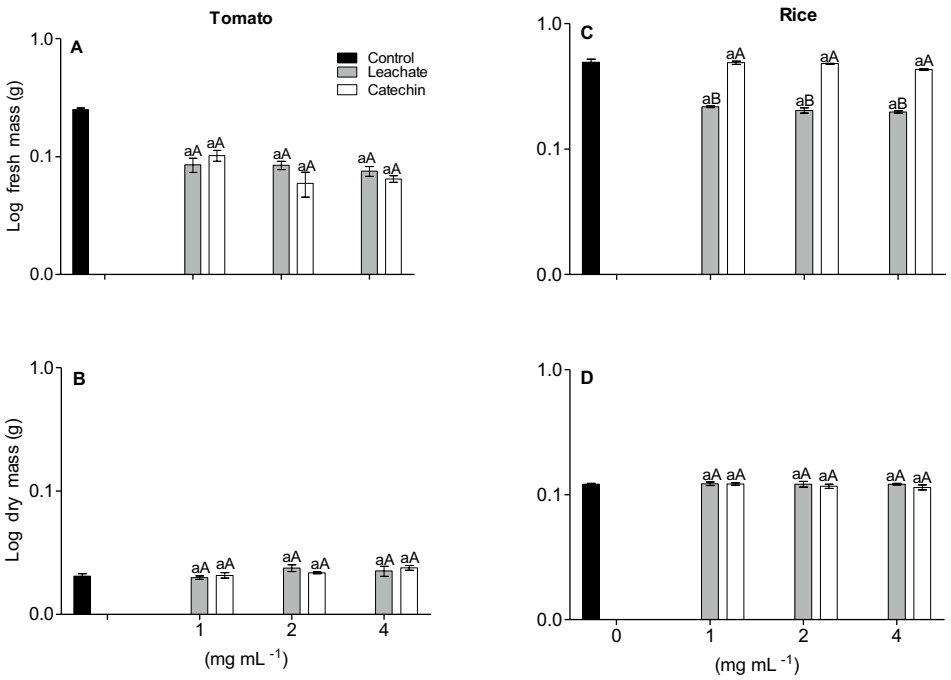

Fig. 2 Effect of different concentrations of seed leachates of *Sesbania virgata* and commercial catechin on the fresh and dry mass of tomato (**A** and **C**, respectively) and rice (**C** and **D**, respectively) after four days of imbibition. The bars indicate the standard error ($N=3$). Lower case letters compare concentrations within each treatment and upper case letters between treatments. Values with the same letters are not significantly different by Tukey's test ($P<0.05$)

Table 1 The effect of co-germination of tomato and rice with *Sesbania virgata* for 5 days on the germination of tomato and rice seeds (G), their germination speed index (GSI), length of root and shoot, and fresh and dry mass of whole seedling (including endosperm)

Treatments	% G	GSI	Length (cm) Mass (mg)			
			Root	Shoot	Fresh	Dry
Tomato (control)	100.0 ± 0.0 a	3.1 ± 0.1 a	3.58 ± 1.07 a	1.61 ± 0.52 a	81.1 ± 10.1 a	12.0 ± 1.4 a
Tomato + *S. virgata*	46.6 ± 1.5 b	0.9 ± 0.5 b	0.1 ± 0.22 b	0.04 ± 0.03 b	30.8 ± 7.0 b	14.0 ± 0.1 a
Rice (control)	66.6 ± 0.0 a	1.2 ± 0.3 a	0.88 ± 0.06 a	0.35 ± 0.10 a	109.9 ± 9.8 a	65.1 ± 8.4 a
Rice + *S. virgata*	77.7 ± 1.5 a	1.7 ± 0.1 a	0.21 ± 0.23 b	0.05 ± 0.09 b	104.4 ± 4.5 a	71.0 ± 1.6 a

Means ± standard deviation ($N=3$). Values followed by the same letters are not significantly different by Tukey's test ($P<0.05$). Letters compare means between treatments for each plant tested.

% G percentage of germination, *GSI* germination speed index

Table 2 Germination (%) of tomato and rice seeds with aqueous seed leachates of *S. virgata* or commercial catechin at 1, 2 or 4 mg mL^{-1}

Germination (%)								
Day	Leachate (mg mL^{-1})				Catechin (mg mL^{-1})			
	0 (control)	1	2	4	0 (control)	1	2	4
Tomato								
2	60.0 ± 10.0 a	23.3 ± 5.7 b	0.8 ± 0.0 c	0.8 ± 0.0 c	33.3 ± 3.2 a	43.3 ± 2.5 a	36.7 ± 2.8 a	36.7 ± 1.5 a
3	80.0 ± 0.0 a	25.0 ± 5.0 b	3.3 ± 5.7 d	10.0 ± 17.3c	63.3 ± 2.5 a	63.3 ± 1.5 a	83.3 ± 0.5 a	73.3 ± 1.1 a
4	83.3 ± 5.7 a	25.0 ± 15.2 b	3.3 ± 5.7 d	20.0 ± 10.0 c	70.0 ± 3.0 a	66.7 ± 1.5 a	86.7 ± 1.1 a	76.7 ± 1.5 a
5	86.7 ± 5.7 a	26.0 ± 5.2 b	10.0 ± 10.0 c	26.7 ± 20.0b	83.3 ± 2.0 a	76.7 ± 0.5 a	86.7 ± 1.1 a	83.3 ± 0.5 a
Rice								
2	26.7 ± 11.5 a	13.3 ± 11.5 b	20.0 ± 20.0 b	0.0 ± 0.0 c	60.0 ± 1.0 a	6.7 ± 0.5 c	0.0 ± 0.0 d	33.3 ± 1.1 e
3	93.3 ± 11.5 a	100.0 ± 0.0 a	53.3 ± 11.5 b	80.0 ± 0.0 a	100.0 ± 0.0 a	93.3 ± 0.5 a	86.7 ± 0.5 a	80.0 ± 1.0 a
4	100.0 ± 0.0 a	100.0 ± 0.0 a	80.0 ± 20.0 a	93.3 ± 11.5 a	100.0 ± 0.0 a	100.0 ± 0.0 a	93.3 ± 0.5 a	86.7 ± 1.0 a
5	100.0 ± 0.0 a	100.0 ± 0.0 a	86.7 ± 11.5 a	93.3 ± 11.5 a	100.0 ± 0.5 a	100.0 ± 0.0 a	100.0 ± 0.0 a	86.7 ± 1.1 a

The control (0) refers to seeds germinated with distilled water. Means ± standard deviation ($N=3$). Values followed by the same letters are not significantly different by Tukey's test ($P^{<}$ 0.05). Letters compare different concentrations at same day for each treatment (leachate or catechin)

(Table 2). In the present study, the catechin detected in the leachates of *S. virgata* seeds using the vanillin method at 279 µg mL^{-1}, corresponded to 30% of the concentration of the commercial catechin (1 mg mL^{-1}) used in phytotoxicity bioassays.

The effect of *S. virgata* leachates and catechin on rice germination was observed only on the 2nd day after the beginning of the imbibition at all concentrations, indicating a delay rather than an inhibitory effect. After the 3rd day, germination of rice seeds was less affected by the leachates than that of tomato seeds (Table 2). Considering that osmotic effects might lead to an overestimation of allelopathic effects, we measured the osmolarity of the solutions and performed assays using polyethylene glycol (PEG 6000). Our results indicated that low osmotic potentials do not explain the inhibitory effects observed on tomato and rice (data not shown).

The aqueous leachate of *S. virgata* and the catechin concentrations from 1 mg mL^{-1} significantly affected the root and shoot lengths of both tomato and rice seedlings (Fig. 1). However, no differences between doses were found. The strongest effects were observed on the shoot and root lengths of tomato germinated with *S. virgata* leachates compared with rice (Fig. 1A, C). Catechin, in turn, severely inhibited root growth (Fig. 1B), but it was less inhibitory to shoot growth in rice (Fig. 1D).

The fresh mass of tomato showed a significant reduction in response to *S. virgata* leachates and catechin than control plants (Fig. 2A). In rice seeds, we found a significant reduction in seedling fresh mass only with leachates of *S. virgata* independently of the concentration assayed (Fig. 2B). The presence of leachates and catechin did not affect the dry mass of tomato and rice seedlings (Fig. 2C, D).

Based on the results described above, we selected 1 mg mL^{-1} of leachates or catechin as the adequate concentration to conduct the following experiments. Figure S2 of the supplementary material shows the aspect of tomato and rice seedlings after 4 days of germination with 1 mg mL^{-1} leachates of *S. virgata* and catechin compared to the control.

Effect of *S. virgata* seed leachates on the degradation of storage carbohydrates

Control tomato seeds germinated in distilled water showed a significant reduction in the soluble sugar content after the 2nd day of seed imbibition (Fig. 3A), which was concomitant with the increase in the concentration of reducing sugars (Fig. 3B). In contrast, the concentrations of total sugars remained high up to the 3rd day in tomato seeds germinated with *S. virgata* leachates or catechin (Fig. 3A). As shown in Fig. 3B, no increase in the reducing sugar concentration was observed in the treatment with seed leachates. Catechin,

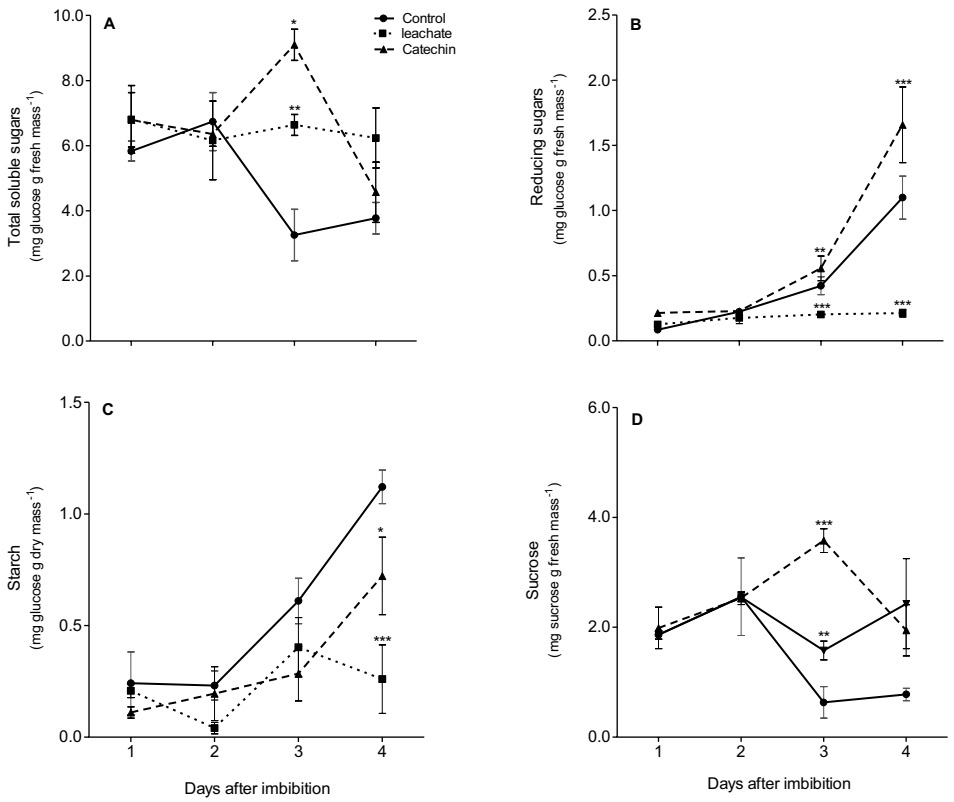

Fig. 3 Total sugar (**A**), reducing sugars (**B**), starch (**C**), and sucrose (**D**) content of tomato during four days of germination with 1 mg $mL.^{-1}$ of *S. virgata* leachate or commercial catechin. Bars indicate standard error ($N=3$). The asterisks indicate differences of statistical significance between treatments by Bonferroni post hoc test $***P<0.001$, $**P<0.01$, $*P<0.05$

in turn, promoted a higher increase in the concentration of reducing sugars than the control.

In control tomato seeds, we found an increase in the starch content after the 2nd day. Seeds treated with leachates or catechin also showed an increase at the 4th day, but it was significantly lower than in control seeds (Fig. 3C). A significant accumulation of sucrose at the 3rd day was observed in seeds treated with *S. virgata* leachates and with catechin compared to the control (Fig. 3D), which is consistent with a high content of total sugar observed at day 3 (Fig. 3A).

The total sugar content in rice increased after the 2nd day of germination in distilled water or with catechin. Still, the same increase did not occur in seeds germinated with *S. virgata* leachates (Fig. 4A). Reducing sugar content represented a small proportion of total sugars in rice seeds (Fig. 4B). An increase was observed from the beginning of the imbibition in both the control and the seeds germinated with catechin. In contrast, in the presence of *S. virgata* leachates, we observed only a slow increase at day 4 (Fig. 4B). Both starch and sucrose content of rice seeds showed no significant changes between treatments (Fig. 4C, D). Although the starch values increased in the control seeds from day 1 to 4, this increase was not statistically significant.

In both species, the activities of α-galactosidase and α-amylase were not significantly different between treatments after 4 days of imbibition (Table 3 and 4). In rice, the endo-β-mannanase and vacuolar acid invertase also did not have differences between treatments (Tables 3 and 4), whereas in tomato the endo-β-mannanase activity was significantly inhibited by leachates and stimulated by catechin (Table 3). The vacuolar acid invertase in tomato seeds germinated with the leachates of *S. virgata* was strongly stimulated, whereas it was reduced in those germinated with catechin compared to the control (Table 4).

Discussion

Sesbania virgata seeds negatively affected germination, seedling growth, and/or carbohydrate metabolism of the target crop species in experiments of co-germination. Our previous studies performed in vitro (Veronesi 2013) and under field conditions (El Id et al. 2015) have shown that seeds of *S. virgata* exert a negative effect on germination and growth of species co-occurring in the surrounding vegetation. Recently, we also demonstrated that *Leucaena leucocephala*, an exotic invasive legume species in Brazil, had the germination rates, seedling growth, and galactomannan metabolism negatively affected by seeds of *S. virgata* (Mignoni et al. 2017). Allelopathy has also been documented in other seeds, including *Lupinus albus, Coffea arabica*, *Camellia sinensis* and *Ipomoea tricolor* (Wink 1983; Suzuki and Waller 1987; Macías-Rubalcava et al. 2008). In the present work, the decrease in tomato germination rates and the seedling

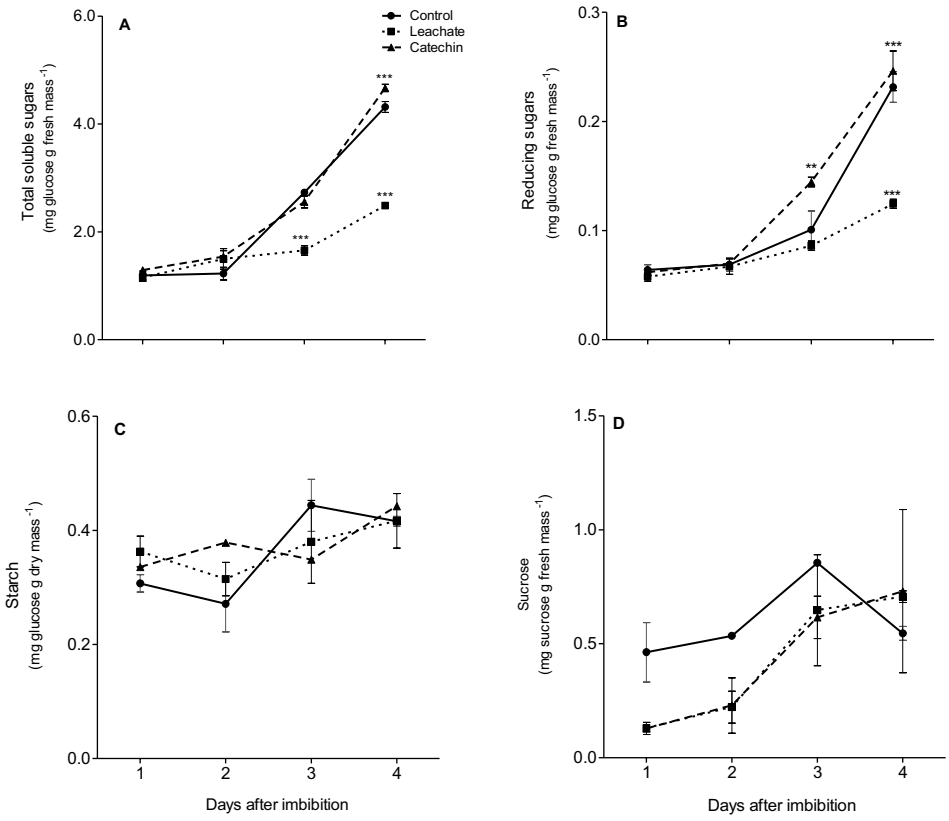

Fig. 4 Total sugar (**A**), reducing sugars (**B**), starch (**C**), and sucrose (**D**) of rice during four days of germination with 1 mg mL^{-1} of *S. virgata* leachate or commercial catechin. Bars indicate the standard error ($N=3$). The asterisks indicate differences of statistical significance between treatments by Bonferroni post hoc test $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$

Table 3 Activity of α-galactosidase and endo-β-mannanase in seeds of tomato and rice after four days of imbibition with distilled water (control), leachate of *S. virgata* seeds or commercial catechin (1 mg mL^{-1})

Species	Treatment	α- Galactosidase		Endo β-mannanase	
		Activity ($U\ min^{-1}$)[A]	Specific activity ($U\ mg^{-1}$ protein)	Activity ($U\ min^{-1}$)[B]	Specific activity ($U\ mg^{-1}$ protein)
Tomato	Control	2.63 ± 0.26	0.015 ± 0.002	0.57 ± 0.02 b	0.53 ± 0.04 b
	Leachate	1.82 ± 0.45	0.009 ± 0.002	0.22 ± 0.04 c	0.14 ± 0.01 c
	Catechin	2.44 ± 0.76	0.013 ± 0.003	0.69 ± 0.06 a	0.68 ± 0.07 a
Rice	Control	0.47 ± 0.04	0.004 ± 0.000	0.38 ± 0.03 a	0.22 ± 0.08 a
	Leachate	0.34 ± 0.04	0.003 ± 0.000	0.32 ± 0.02 a	0.14 ± 0.06 a
	Catechin	0.41 ± 0.06	0.003 ± 0.001	0.40 ± 0.04 a	0.24 ± 0.03 a

Means ± standard deviation ($N = 3$). Values followed by the same letters are not significantly different by Tukey's test ($P < 0.05$). Letters compare means between treatments for each plant tested

[A]An enzyme unit (U) was defined as the amount of enzyme necessary to produce 1 µmol galactose per minute of reaction ($U\ min^{-1}$)

[B]An enzyme unit (U) was defined according to β-Mannazyme tablets (Megazyme, batch 50,201)

Table 4 Activity of α-amylase and vacuolar acid invertase in tomato and rice seeds after four days of imbibition with distilled water (control), leachates of *S. virgata* seeds or commercial catechin (1 mg mL^{-1})

Species	Treatment	α-Amylase		Vacuolar invertase
		Activity ($U\ min^{-1}$)[A]	Specific activity ($U\ mg^{-1}$ protein)	Specific activity ($U\ min^{-1}$)[B]
Tomato	Control	5.13 ± 2.35 a	0.07 ± 0.02 a	0.049 ± 0.045 b
	Leachate	9.76 ± 6.79 a	0.10 ± 0.07 a	0.123 ± 0.003 a
	Catechin	6.39 ± 0.38 a	0.09 ± 0.01 a	0.023 ± 0.001 c
Rice	Control	18.14 ± 7.78 a	0.35 ± 0.21 a	0.113 ± 0.014 a
	Leachate	25.52 ± 0.01 a	0.43 ± 0.01 a	0.110 ± 0.035 a
	Catechin	14.94 ± 5.25 a	0.31 ± 0.20 a	0.154 ± 0.024 a

Means ± standard deviation ($N = 3$). Values followed by the same letters are not significantly different by Tukey's test ($P < 0.05$). Letters compare means between treatments for each plant tested

[A]An enzyme unit (U) was defined as the amount of enzyme needed to produce 1 µmol maltose per minute of reaction ($U\ min^{-1}$)

[B]An enzyme unit (U) was defined as the amount of enzyme required to produce 1 µmol glucose per minute of reaction ($U\ min^{-1}$)

growth inhibition of both tomato and rice caused just by one seed of *S. virgata* clearly demonstrated its seed-to-seed phytotoxic effect on these species. *Sesbania virgata* is considered as an invasive species (Andrade 2006; Souza et al. 2011) and, therefore, these phytotoxic effects might be a potentially important strategy to increase seedling survival at the life beginning.

Although *S. virgata* negatively impacted both tomato and rice, these crops presented a distinct sensibility to its seeds. In tomato, the co-germination with *S. virgata* caused inhibition of germination and a decrease in fresh mass when compared with the control (Table 1). Therefore, it is reasonable to suggest that the differences in tomato germination caused by *S. virgata* could be related to a difference in the imbibition process, in which a delay in water uptake would compromise radicle protrusion. The water-soluble leached allelochemicals likely drive the inhibitory effects since we observed that this inhibition persisted throughout the experiment performed with isolated seed leachates. The impairment of the reserve metabolism caused by *S. virgata* leachates can also be an effect of the low water uptake. Accumulation of sucrose together with the absence of changes in the reducing sugar content observed 3 days after imbibition in tomato seeds (Fig. 3) indicated delayed soluble sugars mobilisation. These findings are consistent with lower starch accumulation in leachate-treated seeds compared with control.

Mannans are the main carbohydrate reserve (> 60%) stored in the endosperm cell walls of tomato seeds (Chen and Bradford 2000). The endosperm, which completely encloses the embryo, represents the predominant constraining structure for radicle emergence. The enzyme weakening of the endosperm after imbibition is required to allow the complete radicle protrusion (Sitrit et al. 1999; Nonogaki et al. 2000). Endo-β-mannanases, polygalacturonases, and glucanases have been associated with this process and with the mobilisation of mannans during and after germination

(Nonogaki et al. 2000). The activity of endo-β-mannanase was significantly lower in tomato seeds treated with *S. virgata* leachates than in the control, suggesting that the inhibition of the endosperm weakening might also be related to the delay of radicle emergence. In seeds that store galactomannans or mannans, there is an increase in the level of starch due to the endosperm carbohydrate mobilisation after germination, providing monosaccharides, which are transported from this tissue to the embryo and then stored (Buckeridge and Dietrich 1996), as observed in water-germinated tomato seeds (Fig. 3). Therefore, the lower starch content observed in the tomato leachate-treated seeds than in the control evidenced a slow endosperm disassembling (Fig. 3C). Reduced endo-β-mannanase activity due to stress by allelochemicals was previously reported in tomato seeds treated with leaf leachates of *Sycios deppei*. The low activity of this cell-wall degrading enzyme was correlated with low levels of gene expression and with a delay in radicle protrusion (Lara-Núñez et al. 2009).

In tomato seeds, α-galactosidase is found both in the cell wall as well as in the cytoplasm and is implicated in the degradation of storage carbohydrates during and after germination (Feurtado et al. 2001), being referred as responsible for integument softening. α-Galactosidase is also involved in the degradation of cell wall mannans (Bassel et al. 2001). In the present study, the α-galactosidase activity values found in tomato seeds were higher than in rice seeds, but no significant differences were observed between treatments, which suggests the absence of a direct effect of allelochemicals from *S. virgata* seeds, including (+)—catechin, on the activity of this enzyme.

Although catechin is leached in high concentrations (235 µg of catechin/per seed) from seeds of *S. virgata* after 24 h of imbibition (Simões et al. 2008), the commercial catechin did not affect tomato germination independently of the concentration. Moreover, tomato seeds treated with catechin showed a significant increase in the activity of endo-β-mannanase compared to the control (Table 2). These findings suggest that other bioactive substances present in the seed leachates, acting either alone or synergistically, rather than catechin, might cause the inhibitory effects on this target crop species. Indeed, high amounts of abscisic acid (Tonini et al. 2006), and the phytotoxic alkaloid sesbanimida A (Powell et al. 1990; Van Staden and Grobbelaar 1995) were reported in the seed leachates and the seed coat of *Sesbania species*. In a previous study, we demonstrated that 2.4 nmoles seed^{-1} of ABA are leached from *S. virgata* seeds at the beginning of imbibition process, decreasing significantly until the fourth day, when it was absent in the leachates (Mignoni et al. 2018). ABA is a potent inhibitor of seed germination and considered an important allelochemical (Zhao et al. 2011). It has been shown that it interferes with the activity of galactomannan-hydrolysing, modulating galactomannan degradation in seeds of fenugreek (Reid and Meier 1973), tomato (Nomaguchi et al. 1995), carob (Seiler 1977), lettuce (Halmer and Bewley 1979) and *S. virgata* (Potomati and Buckeridge 2002). Our results also indicate negative effects of leachates of *S. virgata* seeds and catechin on starch accumulation during the early development of tomato, probably due to a delay in soluble sugar mobilisation from the endosperm. Starch provides glucose that is used in respiration, energy generation, and to compose physical structures during the embryo growth (Magalhães et al. 2010).

Rice germination was much less sensitive to the seed allelochemical effects or the seed leachates of *S. virgata* than tomato. Olofsdotter et al. (1999) observed that different allelopathic effects in rice could be due to the fact that some cultivars can control weeds since they are capable of releasing substances into the environment that reduce the development of these species. Kato-Noguchi (2004) reported the presence of momilactone B in rice, an allelochemical with potential inhibitory effect on neighbouring plants.

We observed only an initial delay in rice germination with aqueous leachate or catechin (Table 2). However, *S. virgata* leachates have affected soluble sugar metabolism in rice, changing reducing sugar levels compared to the control (Fig. 4B). Interestingly, the acidic vacuolar invertase, which regulates sucrose storage and hydrolysis in growing tissues (Winter and Huber 2000), was not affected (Table 4). The starch content showed no significant changes and corroborated the fact that α-amylase activity was not affected by during four days of germination, independently of the treatment. Cell wall and cytosolic invertase activity in seeds were very low or undetectable (data not shown).

Commercial catechin, as well as seed leachates, negatively affected tomato and rice length of roots and shoots (Fig. 1, Table 1). Indeed, 279 µg mL^{-1} of catechin were detected in *S. virgata* seed leachates. Catechin, as other phenolic phytotoxic compounds, can affect cell ultrastructure and division and root elongation, and therefore, impacts whole plant development (Li et al. 2010). This suggested that the inhibitory effect caused by *S. virgata* seed leachates on these parameters might be related to the presence of this flavonoid.

Conclusions

Our results indicate that the allelochemicals leached from *S. virgata* seeds negatively affect the germination and seedling growth of crop species, probably contributing to its invasive behaviour and its successful colonization in a variety of natural ecosystems and plantations. It is also clear that the inhibitory effects of *S. virgata* cannot be exclusively attributed to the flavonoid catechin leached from its seeds.

Instead, they seem to result from an arsenal of leached allelochemicals with different biological effects, which appear to interfere with the mobilisation of stored soluble and cell-wall carbohydrates.

Author contribution statement MRB designed research, LCSC and DSBM conducted and performed the experiments, LCSC, DSBM, CJB and MRB interpreted the results and DSBM, CJB and MRB wrote the manuscript.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-022-03431-5.

Acknowledgements This work is part of the Ph.D. thesis of L.C.S. Coelho at the Post-Graduate Program in Cellular and Structural Biology, University of Campinas, SP (Brazil) and was supported by "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Doutorado Interunidades" (CAPES/DINTER) and "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior /Programa de Apoio a Pós-Graduação" (CAPES/PROAP). The authors thank "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, Grant 474325/2009-1) and "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP, Grants 2005/04139-7 and 2012/16332-0), for supporting this work. M.R. Braga thanks CNPq for the research fellowship.

Funding Conselho Nacional de Desenvolvimento Científico e Tecnológico, 474325/2009-1, Marcia Regina Braga, Fundação de Amparo à Pesquisa do Estado de São Paulo, 2005/04139-7, Marcia Regina Braga, 2012/16332-0, Marcia Regina Braga.

Declarations

Conflict of interest Authors declare that there is no conflict of interest.

References

Amaral LI, Gaspar M, Felix Costa PM, Pereira M, Aidar M, Silveira Buckeridge M (2007) Novo método enzimático rápido e sensível de extração e dosagem de amido em materiais vegetais. Hoehnea 4:425–431. https://doi.org/10.1590/S2236-89062007000400001

Andrade LA (2006) Espécies exóticas invasoras no nordeste do Brasil: impactos nos ecossistemas locais. In: Mariath JEA, Santos RP (eds) Os avanços da Botânica no início do século XXI: Morfologia Fisiologia, Taxonomia, Ecologia e Genética. Sociedade Botânica do Brasil, Porto Alegre, pp 524–528

Bassel GW, Mullen RT, Bewley JD (2001) α-Galactosidase is synthesized in tomato seeds during development and is localized in the protein storage vacuoles. Can J Bot 79:1417–1424. https://doi.org/10.1139/cjb-79-12-1417

Bernfeld P (1955) Amylase α and β. Methods Enzymol 1:149–158. https://doi.org/10.1016/0076-6879(55)01021-5

Blair AC, Hanson BD, Brunk GR, Marrs RA, Westra P, Nissen SJ, Hufbaueret RA (2005) New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecol Lett 8:1039–1047. https://doi.org/10.1111/j.1461-0248.2005.00805.x

Blair AC, Nissen SJ, Brunk GR, Hufbauer RA (2006) A lack of evidence for an ecological role of the putative allelochemical (±)-catechin in spotted knapweed invasion success. J Chem Ecol 32:2327–2331. https://doi.org/10.1007/s10886-006-9168-y

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Branzini A, González RS, Zubillaga M (2012) Absorption and translocation of copper, zinc and chromium by *Sesbania virgata*. J Environ Manage 102:50–54. https://doi.org/10.1016/j.jenvman.2012.01.033

Broadhurst RB, Jones WT (1978) Analysis of condensed tannins using acidified vanillin. J Sci Food Agric 29:788–794. https://doi.org/10.1002/jsfa.2740290908

Buckeridge MS, Dietrich SMC (1996) Mobilisation of the raffinose family oligosaccharides and galactomannan in germinating seeds of *Sesbania marginata* Benth. (Leguminosae-Faboideae). Plant Sci 117:33–43. https://doi.org/10.1016/0168-9452(96)04410-X

Caccere R, Teixeira SP, Centeno DC, Figueiredo-Ribeiro RCL, Braga MR (2013) Metabolic and structural changes during early maturation of *Inga vera* seeds are consistent with the lack of a desiccation phase. J Plant Physiol 170:791–800. https://doi.org/10.1016/j.jplph.2013.01.002

Carvalho CP, Hayashi AH, Braga MR, Nievola CC (2013) Biochemical and anatomical responses related to the in vitro survival of the tropical bromeliad *Nidularium minutum* to low temperatures. Plant Physiol Biochem 71:144–154. https://doi.org/10.1016/j.plaphy.2013.07.005

Chen F, Bradford KJ (2000) Expression of an expansin is associated with endosperm weakening during tomato seed germination. Plant Physiol 124:1265–1274. https://doi.org/10.1104/pp.124.3.1265

Chobot V, Huber C, Trettenhahn G, Hadacek F (2009) (±)-Catechin: chemical weapon, antioxidant, or stress regulator? J Chem Ecol 35:980–996. https://doi.org/10.1007/s10886-009-9681-x

De Araujo EC, Mendonça AVR, Barroso DG, Lamônica KR, Silva RF (2004) Caracterização morfológica de frutos, sementes e plântulas de *Sesbania virgata* (Cav.) Pers. Rev Bras Sementes 26:105–110. https://doi.org/10.1590/s0101-31222004000100016

de Oliveira Mello JI, Barbedo CJ, Salatino A, Figueiredo-Ribeiro RCL (2010) Reserve carbohydrates and lipids from the seeds of four tropical tree species with different sensitivity to desiccation. Brazilian Arch Biol Technol 53:889–899. https://doi.org/10.1590/S1516-89132010000400019

Dubois M, Gilles KA, Hamilton JK, Robers PA, Smith J (1956) Colorimetric method for determination of sugars and related substances. Analyt Chem 28:350–356. https://doi.org/10.1021/ac60111a017

El Id VL, da Costa BV, Mignoni DSB, Veronesi MB, Simões K, Braga MR, Santos Junior NAS (2015) Phytotoxic effect of *Sesbania virgata* (Cav.) Pers. on seeds of agronomic and forestry species. J for Res 26:339–346. https://doi.org/10.1007/s11676-015-0026-z

Feurtado JA, Banik M, Bewley JD (2001) The cloning and characterization of α-galactosidase present during and following germination of tomato (*Lycopersicon esculentum* Mill.) seed. J Exp Bot 52:1239–1249. https://doi.org/10.1093/jxb/52.359.1239

Halmer P, Bewley JD (1979) Mannanase Production by the Lettuce Endosperm. Planta 340:333–340. https://doi.org/10.1007/BF00391576

He WM, Feng Y, Ridenour WM et al (2009) Novel weapons and invasion: Biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (±)-catechin. Oecologia 159:803–815. https://doi.org/10.1007/s00442-008-1234-4

Kato-Noguchi H (2004) Allelopathic substance in rice root exudates: rediscovery of momilactone B as an allelochemical. J Plant Physiol 161:271–276. https://doi.org/10.1078/0176-1617-01188

Kissmann KG, Groth D (1999) Infesting and noxious plants, 1st edn. Brazilian BASF, São Paulo, pp 770–776

Lara-Núñez A, Sánchez-Nieto S, Luisa Anaya A, Cruz-Ortega R (2009) Phytotoxic effects of *Sicyos deppei* (Cucurbitaceae) in germinating tomato seeds. Physiol Plant 136:180–192. https://doi.org/10.1111/j.1399-3054.2009.01228.x

Leduc S, Motta N, Silva JPN, Gaspar M, Barbedo CJ, Figueiredo-Ribeiro RCL (2012) Non-structural carbohydrates of immature seeds of *Caesalpinia echinata* (Leguminosae) are involved in the induction of desiccation tolerance. Aust J Bot 60:42–48. https://doi.org/10.1071/BT11236

Li ZH, Wang Q, Ruan X et al (2010) Phenolics and plant allelopathy. Molecules 15:8933–8952. https://doi.org/10.3390/molecules15128933

Lorenzi H (2000) Plantas daninhas do Brasil, 3rd edn. Instituto Plantarum, Nova Odessa, p 640

Luo R, Song X, Li Z et al (2018) Effect of soil salinity on fructan content and polymerization degree in the sprouting tubers of Jerusalem artichoke (*Helianthus tuberosus* L.). Plant Physiol Biochem 125:27–34. https://doi.org/10.1016/j.plaphy.2018.01.025

Ma X, Guo J, Han X, Yan G (2015) Grevillea (Proteaceae) seed coats contain inhibitors for seed germination. Aust J Bot 63:566–571. https://doi.org/10.1071/BT15085

Macías-Rubalcava ML, HernándezBautista BE, Anaya AL (2008) Production of allelopathic glycosidic resins in seeds and early development stages of *Ipomoea tricolor* L. (Convolvulaceae). Allelopath J 21:107–118

Magalhães SR, Borges EEL, Berger APA (2010) Mobilização de reservas no eixo embrionário e nos cotilédones de sementes de *Schizolobium parahyba* (vell.) S. F. Blake Durante a Germinação Cienc Florest 20:589–595. https://doi.org/10.5902/198050982417

Maguire JD (1962) Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Sci 2:176–177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Mignoni DSB, Cabral RS, Torres LMB, Braga MR (2017) Phytotoxic effects of *Leucaena leucocephala* seeds on seeds germination and seedling growth of rice and tomato. Allelopathy J 42:279–294. https://doi.org/10.26651/allelo.j/2017-42-2-1123

Mignoni DSB, Simões K, Braga MR (2018) Potential allelopathic effects of the tropical legume *Sesbania virgata* on the alien *Leucaena leucocephala* related to seed carbohydrate metabolism. Biol Invasions 20:165–180. https://doi.org/10.1007/s10530-017-1524-z

Ndakidemi PA, Dakora FD (2003) Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. Funct Plant Biol 30:729–745. https://doi.org/10.1071/FP03042

Nomaguchi M, Nonogaki H, Morohashi Y (1995) Development of galactomannan-hydrolyzing activity in the micropylar endosperm tip of tomato seed prior to germination. Physiol Plant 94:105–109. https://doi.org/10.1111/j.1399-3054.1995.tb00790.x

Nonogaki H, Gee OH, Bradford KJ (2000) A germination-specific endo-β-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. Plant Physiol 123:1235–1245. https://doi.org/10.1104/pp.123.4.1235

Olofsdotter M, Watson A, Piggin C (1999) Allelopathy in rice. In: Hassan SM (ed) Weed management using allelopatic rice varieties in Egypt. International Rice Research Institute, Manilla, Philippines, pp 27–37

Potomati A, Buckeridge MS (2002) Effect of abscisic acid on the mobilisation of galactomannan and embryo development of *Sesbania virgata* (Cav.) Pers. (Leguminosae - Faboideae). Rev Brasil Bot 25:303–310. https://doi.org/10.1590/s0100-84042002000300006

Pott A, Pott VJ (1994) Plantas do Pantanal, EMBRAPA/CPAP/SPI, Corumbá

Powell R, Plattner R, Suffness M (1990) Occurrence of sesbanimide in seeds of toxic *Sesbania species*. Weed Sci 38:148–152. https://doi.org/10.1017/S0043174500056290

Reid JS, Meier H (1973) Enzymic activities and galactomannan mobilisation in germinating seeds of fenugreek (*Trigonella foenum-graecum* L. Leguminosae). Planta 112:301–308. https://doi.org/10.1007/bf00390303

Seiler A (1977) Galactomannan breakdown in germinating carob seeds (*Ceratonia siliqua* L.). Planta 134:209–221. https://doi.org/10.1007/BF00384185

Shimon-Kerner N, Mills D, Merchuk JC (2000) Sugar utilization and invertase activity in hairy-root and cell-suspension cultures of *Symphytum officinale*. Plant Cell T Organ Cult 62:89–94. https://doi.org/10.1023/A:1026506426420

Simões K, Du J, Kretzschmar FS, Broeckling CD, Stermitz FS, Vivanco JM, Braga MR (2008) Phytotoxic catechin leached by seeds of the tropical weed *Sesbania virgata*. J Chem Ecol 34:681–687. https://doi.org/10.1007/s10886-008-9443-1

Sitrit Y, Hadfield KA, Bennett AB, Bradford KJ, Downie AB (1999) Expression of a polygalacturonase associated with tomato seed germination. Plant Physiol 121:419–428. https://doi.org/10.1104/pp.121.2.419

Somogyi M (1945) A new reagent for determination of sugars. J Biol Chem 160:61–68. https://doi.org/10.1016/S0021-9258(18)43097-9

Souza VC, Andrade LA, Bezerra FTC, Fabricante JR, Feitosa RC (2011) Avaliação populacional de *Sesbania virgata* (Cav.) Pers. (Fabaceae Lindl.), nas margens do rio Paraíba. Rev Bras Ciencias Agrar 6:314–320. https://doi.org/10.5039/agraria.v6i2a926

Souza Filho APS (2002) Atividade potencialmente alelopática de extratos brutos e hidroalcoólicos de feijão-de-porco (*Canavalia ensiformis*). Planta Daninha 20:357–364. https://doi.org/10.1590/s0100-83582002000300005

Souza Filho APS, Trezzi MM, Ioue MH (2011) Sementes como fonte alternativa de substâncias químicas com atividade alelopática. Planta Daninha 29:709–716. https://doi.org/10.1590/S0100-83582011000300025

Suman A, Shahi HN, Singh P, Gaur A (2002) Allelopathic influence of *Vigna mungo* (black gram) seeds on germination and radical growth of some crop plants. Plant Growth Regul 38:69–74. https://doi.org/10.1023/A:1020943011207

Suzuki T, Waller GR (1987) Allelopathy due to purine alkaloids in tea seeds during germination. Plant Soil 98:131–136. https://doi.org/10.1007/BF02381733

Thorpe AS, Thelen GC, Diaconu A, Callaway RM (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. J Ecol 97:641–645. https://doi.org/10.1111/j.1365-2745.2009.01520.x

Tonini PP, Lisboa CGS, Freschi L, Mercier H, Mazzoni-Viveiros SC, Buckeridge MS (2006) Effect of abscisic acid on galactomannan degradation and endo-β-mannanase activity in seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae). Trees - Struct Funct 20:669–678. https://doi.org/10.1007/s00468-006-0082-2

Tonini PP, Purgatto E, Buckeridge MS (2010) Effects of abscisic acid, ethylene and sugars on the mobilization of storage proteins and carbohydrates in seeds of the tropical tree *Sesbania virgata* (Leguminosae). Ann Bot 106:607–616. https://doi.org/10.1093/aob/mcq159

Van Staden J, 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. https://doi.org/10.1016/0098-8472(95)00005-0

Veronesi MB (2013) Avaliação da tolerância de duas espécies nativas às fitotoxinas exsudadas por *Sesbania virgata* (Cav.) Pers. Dissertation, Institute of Botany of São Paulo

Villagrasa M, Guillamón M, Labandeira A, Taberner A, Eljarrat E, Barceló D (2006) Benzoxazinoid allelochemicals in wheat: distribution among foliage, roots, and seeds. J Agric Food Chem 54:1009–1015. https://doi.org/10.1021/jf050898h

Wang CM, Li TC, Jhan YL, Weng J-H, Chou C-H (2013) The impact of microbial biotransformation of catechin in enhancing the allelopathic effects of *Rhododendron formosanum*. PLoS ONE 8:1–14. https://doi.org/10.1371/journal.pone.0085162

Weidlich EWA, Pescador R, Uhlmann A (2010) Alocação de recursos (carboidratos) no desenvolvimentoinicial de plântulas de *Schizolobium parahyba* (vell.) s.f. Blake (Fabaceae - Caesalpinioideae). Rev Arvore 34:627–635. https://doi.org/10.1590/S0100-67622010000400007

Wink M (1983) Inhibition of seed germination by quinolizidine alkaloids - aspects of allelopathy in *Lupinus albus* L. Planta 158:365–368. https://doi.org/10.1007/BF00397339

Winter H, Huber SC (2000) Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. CRC Crit Rev Plant Sci 19:31–67. https://doi.org/10.1080/07352680091139178

Zahedi SM, Ansari NA (2011) Allelopathic potential of common mallow (*Malva sylvestris*) on the germination and the initial growth of tomato, cucumber and cress. Asian J Agric Sci 3:235–241

Zhang S, Liu J, Bao X, Niu K (2011) Seed-to-seed potential allelopathic effects between *Ligularia virgaurea* and native grass species of Tibetan alpine grasslands. Ecol Res 26:47–52. https://doi.org/10.1007/s11284-010-0751-x

Zhang Z, Liu Y, Yuan L, Weber E, van Kleunen M (2021) Effect of allelopathy on plant performance: a meta-analysis. Ecol Lett. https://doi.org/10.1111/ele.13627

Zhao H, Peng S, Chen Z, Wu Z, Zhou G, Wang X, Qiu Z (2011) Abscisic acid in soil facilitates community succession in three forests in china. J Chem Ecol 37:785–793. https://doi.org/10.1007/s10886-011-9970-z