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Germination and initial development of forest species under the action of catechin, presents in seeds of *Sesbania virgata* (Cav.) Pers. (Fabaceae)[1]

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ABSTRACT – (Germination and initial development of forest species under the action of catechin, presents in seeds of *Sesbania virgata* (Cav.) Pers. (Fabaceae)). *Sesbania virgata* is a shrub that occurs in riparian forests, especially in Cerrado and Atlantic Forest. It is considered superdominant due to its rapid growth and high soil cover. Its seeds release allelochemicals during imbibition, affecting the germination and initial growth of other species. The aim of this study was to evaluate the phytotoxic effect of compounds found in *S. virgata* seeds on co-occurrent species from different successional stages. The species were co-germinated with *S. virgata* seeds and irrigated with *S. virgata* integument extracts, in laboratory, greenhouse, and field. The germination rate, germination speed, germination speed index, and seedling growth of the species were evaluated. Phytochemicals released by *S. virgata* seeds were not able to inhibit the germination of the co-occurrent forest species in field, but significantly reduced their initial growth. The results suggest that resistance to allelochemicals is not linked to the successional stage of a species but is determined by specific characteristics that guarantee the ability to tolerate the phytotoxins released by the seeds of *S. virgata*. In addition, the results also suggest that catechin and other compounds found in seeds may be responsible for the inhibitory potential of *S. virgata* plants.
Keywords: allelopathy, plant establishment, successional stages, superdominat species

RESUMO – (Germinação e desenvolvimento inicial de espécies florestais sob a ação da catequina, presente nas sementes de *Sesbania virgata* (Cav.) Pers. (Fabaceae)). *Sesbania virgata* é um arbusto que ocorre em matas ciliares, principalmente no Cerrado e Mata Atlântica. É considerada superdominante devido ao rapido crescimento e alta cobertura do solo. Suas sementes liberam aleloquímicos durante a embebição, afetando germinação e crescimento inicial de outras espécies. O objetivo desse estudo foi avaliar o efeito fitotóxico de compostos encontrados em sementes de *S. virgata,* em espécies co-ocorrentes de diferentes estágios sucessionais. As espécies foram co-germinadas com sementes de *S. virgata* e irrigadas com extratos de tegumento de *S. virgata,* em laboratório, casa de vegetação e campo. Foram avaliados a taxa de germinação, a velocidade de germinação, o índice de velocidade de germinação e o desenvolvimento inicial das espécies. Fitoquímicos liberados pelas sementes de *S. virgata* não inibiram a germinação das espécies co-ocorrentes no campo, mas reduziram significativamente o desenvolvimento inicial destas. Os resultados sugerem que a resistência a aleloquímicos não está ligada ao estágio sucessional de uma espécie, mas é determinada por características específicas que garantem a capacidade de tolerar as fitotoxinas liberadas pelas sementes de *S. virgata*. Além disso, os resultados também sugerem que catequina e outros compostos encontrados nas sementes podem garantir o potencial inibitório de *S. virgata.*
Palavras-chave: alelopatia, estabelecimento vegetal, estagios sucessionais, espécie superdominante

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Introduction

Plant populations with high propagation rates and rapid growth create serious ecological problems in natural environments because they form massive monocultures. Successful plant growth in a new habitat depends on individual characteristics of each species, including high photosynthetic efficiency, high growth rate, tolerance to defoliation and herbivory, high capacity for regeneration and reproduction, fast reproductive cycle, high seed yield, and high germination capacity (Barbosa *et al.* 2008). Allelopathy has been recognized as a key biological mechanism in the success of plant species in new environments, providing adaptive advantages for some species and facilitating their establishment and survival (Lorenzo *et al.* 2013, Trezzi *et al.* 2016). Allelopathic mechanisms can influence the competitive ability and dominance of individual plants in plant communities, affecting the recruitment and establishment of native species during succession in reforestation projects (Abhilasha *et al.* 2008, Alford *et al.* 2009, Lorenzo *et al.* 2013).

Both intra- and interspecific interference between plants may be caused by allelochemicals (Reigosa *et al.* 1999, Callaway *et al.* 2005), which are secondary metabolites (Weir *et al.* 2004, Reigosa & Gonzales 2006; Tharayil *et al.* 2009). These substances are found in all plant organs (Anaya 1999) including seeds, which release allelochemicals after the onset of imbibition and can contribute to the invasive behavior of some species (Ens *et al.* 2009, Holzmueller & Jose 2011, Iqbal & Fry 2012).

The hypothesis that allelochemicals produced by invasive species provide a competitive advantage over native species proposes that plants co-occurring with species that exude phytotoxins develop resistance mechanisms to these allelochemicals (Inderjit *et al.* 2008, He *et al.* 2009, Thorpe *et al.* 2009, Weidenhamer & Callaway 2010). However, field studies showing that plant toxic substances accumulate in the environment to levels capable of inhibiting the growth of other plants are lacking (Weidenhamer 2005). The allelopathic effects observed in bioassays performed under controlled conditions are difficult to be extrapolated to natural conditions, since in a controlled condition factors that may interfere in the results are not considered, such as soil and climate , genetic changes and biotic interactions (Inderjit & Weston 2000, Weir *et al.* 2004, Trezzi *et al.* 2016).

Some secondary metabolites with allelopathic potential such as phenolic compounds are found in the seed tegument of plants in the genus *Sesbania* (Fabaceae) and may be quickly released during the imbibition process. In *Sesbania drummondii* (Rydb) Cory, the allelochemical (+)-catechin was found in greater amounts compared to (−)-catechin, whereas the opposite was observed in *Sesbania vesicaria* (Jacq.) Ell. in which (−)-catechin predominates (Ceballos *et al.* 1998).

The application of commercial (+)-catechin to seeds of native and crops species were shown to affect root growth in all tested species (Simões *et al.* 2008, Mignoni *et al.* 2017). The flavonoid (+)-catechin is the main allelochemical released by *Sesbania virgata* (Cav.) Pers. seeds (Simões *et al.* 2008). The substance is produced in the seed coat and released in high concentrations (235 mg/seed) after 24 hours of imbibition. In the same study, *S. virgata* was shown to inhibit seedling growth in *Arabidopsis thaliana* (L.) Heynh. and *Oryza sativa* L.

The wand riverhemp *Sesbania virgata* (Cav.) Pers. (Fabaceae-Faboideae) is a fast-growing pioneer shrub native to South America (Molle & Tiné 2009), and found in south, southeast, and central-west Brazil, mainly in the Atlantic Forest and Cerrado biomes (Florentino *et al.* 2009). The species has fast germination rates, high development index, and high ground cover potential (Coutinho *et al.* 2005, Zanandrea *et al.* 2009). *Sesbania virgata* has been used in the restoration of degraded areas and revegetation of gallery forests (Coelho *et al.* 2019) due to its hardiness, tolerance to low fertility soils, and competitive ability against other plant species (Moreira *et al.* 2006, Florentino *et al.* 2009, Branzini *et al.* 2012). Due to its aggressiveness in natural habitats, *S. virgata* is considered a superdominant plant with invasive behavior (Matos & Pivello 2009, Dalmagro *et al.* 2016).

El Id *et al.* (2015) showed that the germination and initial development of agronomic species were significantly affected by *S. virgata* seed exudates. In contrast, the germination and development of non-pioneer native species, which may co-occur with *S. virgata* in its natural habitat, were only moderately affected. In addition, tests performed with leaf extracts of *S. virgata* showed that the germination rate and initial development of forest and agronomic species were not reduced. These results indicate that seeds rather than leaves and branches are the major source of allelochemicals in *S. virgata*.

Few studies have investigated the effect of *S. virgata* allelochemicals on the seed physiology of other co-occurrent plant species (El Id *et al.* 2015), especially under field conditions. From the hypothesis that invasive species producing allelochemicals would have greater competitive advantages under native species, we hypothesized that native and co-occurring species with native species producing allelochemicals would have, over time, acquired mechanisms capable of tolerating such phytotoxicity, due to the common occurrence history. Thus, to test this hypothesis we investigated, in the laboratory, greenhouse and field, the inhibitory effect of *S. virgata* seeds on the germination and initial growth of species that co-occur with *S. virgata* in its natural habitat, which is a native species with allelopathic potential.

Material and Methods

Biological material - Seeds of *S. virgata* were harvested from three different natural populations located in Lavras, Minas Gerais, Brazil (45°00'25''W 21°13'35''S, 45°00'24''W 21°13'30''S, 45°00'39'' 21°13'11''S and altitude of 830m above sea level). The characteristic forest formation of region can be considered as a transition between Montana and Cerrado Seasonal Semideciduous Forest, widely fragmented and in several seral stages (Pereira *et al.* 2010). The collected seeds were stored in a refrigerator for 30 days, until the beginning of experiments.

To test the potential inhibition of *S. virgata* seeds, three co-occurrent species from different successional stages were selected: *Mimosa bimucronata* (DC.) Kuntze (pioneer), *Peltophorum dubium* (Spreng.) Taub. (light-demanding climax), and *Copaifera langsdorffii* Desf. (shade-tolerant climax). These species were selected based on a floristic survey that identified all species with plant height > 1.2 m located within a 10-m radius of *S. virgata* individuals. Seeds of *M. bimucronata* and *P. dubium* were obtained from stored seed lots of the seed bank at the Seed Research Center, Institute of Botany of São Paulo, and *C. langsdorffii* seeds were collected from trees grown in the Botanical Garden of São Paulo, São Paulo, Brazil.

Co-germination assays with *S. virgata* seeds - To all seeds to germinate homogeneously and thus, for the tests species to come into contact with substances from *S. virgata*, the seeds with coat dormancy were scarified. To overcome seed coat dormancy, *S. virgata*, *P. dubium*, and *C. langsdorffii* seeds (Cruz & Carvalho 2006, Pereira *et al.* 2014, Shreelalitha *et al.* 2015) were hand-scarified with sandpaper (P60). *Mimosa bimucronata* seeds were not scarified due to the absence of an impermeable tegument. Next, *M. bimucronata*, *P. dubium*, and *C. langsdorffii* seeds were disinfected with sodium hypochlorite (10%) for 10 min and washed thoroughly with distilled water. *Sesbania virgata* seeds were not disinfected to not affect their exudates. The *S. virgata* seeds were selected from the mixture of seeds from three matrices of *S. virgata*, which produce catechin.

In the laboratory assay, one seed of *M. bimucronata* was co-germinated with 0 (control), 5, or 10 *S. virgata* seeds in 9-cm Petri dishes containing filter paper moistened with 5 mL of distilled water (El Id *et al.* 2015). The control treatment consisted of four replicates, with five Petri dishes per replicate, each plate containing one seed of the tested species, totaling five seeds of each species per replicate, that is, 20 seeds per treatment. This experimental design was applied in co-germination treatments with 5 and 10 *S. virgata* seeds.

The same treatments and conditions were applied for *P. dubium* and *C. langsdorffii*. Plates were maintained in a germination chamber at 25 °C with continuous light (515.87 lux), irrigated with 3–5 mL of distilled water every 48 h, and evaluated daily for 15 days, from the germination of each species. At the end of the experiment, germination rate (G%), germination speed (GS) (Borghetti and Ferreira 2004) and germination speed index (GSI) (Maguire 1962), and root length were evaluated. The same germination treatments described above were used in greenhouse and field conditions, replacing Petri dishes by pots and pits, respectively. In the greenhouse, seeds were sown in pots (415 mL) containing the soil from the place where the field experiments were conducted. The pots were watered daily with 15 mL of distilled water and, after seven days, G%, GS and GSI were determined. The experiment was maintained for 90 days to evaluate plant development, measured as plant height, shoot diameter, and shoot dry weight. Under field conditions, the bioassays were conducted in an area near to Atlantic forest fragments, located at Institue of Botany in São Paulo, SP. Seeds were sown in 15-cm diameter pits and irrigated daily with 15 mL of water. The experiment was evaluated daily and G%, GS and GSI were calculated after seven days, from the germination of each species The field assays were maintained for 90 days for determination of plant height, shoot diameter, and shoot dry weight. The same experimental design used in the laboratory was applied in greenhouse and field treatments

Sesbania virgata seed coat extracts - To produce the aqueous extracts used for irrigation, 1,000 *S. virgata* seeds were scarified as described above and imbibed in 300 mL of distilled water in dark polystyrene plastic boxes (11 × 11 × 3.5 cm) for 8 h at 25 °C. After drying, seed coats were removed from seeds, frozen, freeze-dried, and powdered in a bead mill. The powdered material was suspended in distilled water (1 mg mL^{-1}), shaken on a plate shaker at maximum speed (1500 RPM) for 45 min, filtered, diluted at 0.1, 0.5, and 1.0% (w/v), and used for irrigation. These concentrations were obtained from previous experiments (Simões 2008, El Id *et al.* 2015), and the extracts were produced from the seeds from the matrix that presented higher amount of catechin. In addition to distilled water, an aqueous solution of 1 mg mL^{-1} (+)-Catechin (Sigma-Aldrich) was used as positive control.

Catechin quantification - For the quantification of catechin from seeds, *S. virgata* seed exudates were collected at six-hour intervals for 48 h (6, 12, 18, 24, 30, 36, 42, and 48 h) as three replicates from 50 seeds per replicate at each time interval. The seeds were scarified as described above, immersed in 250 mL of distilled water in dark polystyrene plastic boxes (11 × 11 × 3.5 cm), and placed into climate chamber at 25 °C. Seed exudates were collected every 6 h and stored in a freezer until analysis. The presence of catechin was confirmed after solvent partition of the extracts with ethyl acetate (Simões *et al.* 2008) and subsequent analysis by HPLC. Aliquots of the extracts (20 µL) were analyzed using a C18 column on an Agilent 1220 Infinity

LC DAD high performance liquid chromatographer with a linear gradient of water and methanol acidified with 1% acetic acid (20–100% methanol) at a flow rate of 0.8 mL min^{-1}. (+)-Catechin was used as standard at a concentration of 0.0625, 0.125, 0.025, 0.5, and 1 mg mL^{-1} to obtain a standard curve for comparison with seed extracts (equation: $y = 2676x - 903.3$; R^2: 0.982).

Irrigation assay with *S. virgata* seed coat extracts - Irrigation assays were performed as described above for the co-germination experiments, and seeds of the three tests species were germinated under the same experimental conditions (laboratory, greenhouse, and field), with the same experimental design. *Sesbania virgata* seeds were replaced by aqueous seed coat extracts at 0, 0.1, 0.5, and 1.0%, and a positive control solution of commercial 1 mg mL^{-1} (+)-catechin (1%). In laboratory assays, the seeds were irrigated every 48 h, during the test period, with 3–5 mL of distilled water (control), concentrated seed coat extracts, or catechin, totaling seven irrigation treatments per species. G%, GS and GSI were determined after seven days and root length was measured at the end of the experiment (15 days). In the greenhouse and field assays, seeds and after seedlings were watered daily with 15 mL of distilled water (control), concentrated seed coat extracts, or catechin until the end of the experiment (90 days). After seven days, G%, GS and GSI of the assayed species were calculated and plant height, shoot diameter, and shoot dry weight were recorded at the end of the bioassay. Each of the three experimental conditions included four replicates per treatment with five seeds per replicate.

Experimental design and statistical analysis - Each assay was analyzed as a randomized complete block design using SISVAR 5.1 (Ferreira 2011). Data were analyzed using analysis of variance (ANOVA) and significant differences between treatment means were identified by the Tukey's test ($p < 0.05$).

Results

Germination process - The G% of the species tested was not significantly affected by the number of *S. virgata* seeds in laboratory, greenhouse, or field assays (table 1). However, the GSI of *M. bimucronata* seeds grown in the laboratory with 10 *S. virgata* seeds was significantly lower (0.31) compared to controls (0.45), the opposite being observed in the data referring to the GS, where the highest values were registered with the largest number of seeds. Similarly, the GSI of *P. dubium* seeds grown in the greenhouse with 10 *S. virgata* seeds was significantly lower (0.11) compared to controls (0.15). Regarding the GS data obtained for *M. bimucronata* and *P. dubium* in greenhouse, it was observed that the greater number of seeds caused an increase in the germination speed values.

In the greenhouse irrigation assay, the G% was significantly lower in *M. bimucronata* seeds treated with 0.5 or 1.0% *S. virgata* extracts (table 2), whereas the GSI of *M. bimucronata* seeds treated with *S. virgata* extracts at all concentrations was significantly lower than controls in laboratory and greenhouse assays. In addition, the GSI of *M. bimucronata* seeds treated with catechin extract in greenhouse assays was similar to that of seeds treated with 0.5 or 1.0% *S. virgata* extracts. A significant increase in the GS variable was also observed for individuals of *M. bimucronata* in laboratory and greenhouse conditions, when in contact with a greater number of *S. virgata* seeds.

Initial growth - Root length of *M. bimucronata*, *P. dubium*, and *C. langsdorffii* seedlings decreased significantly with increasing number of *S. virgata* seeds in laboratory germination assays (figure 1 a). The shortest root length for *M. bimucronata* and *C. langsdorffii* seedlings grown with five *S. virgata* seeds was 2.55 and 1.85 cm, respectively, and 2.04 cm for *M. bimucronata* seedlings grown with 10 *S. virgata* seeds. In irrigation assays, mean root lengths of *M. bimucronata* and *P. dubium* seedlings were shorter than those of control seedlings (figure 1 b). In addition, mean root length was shorter in *P. dubium* seedlings treated with catechin extract (2.34 cm) compared to controls (4.88 cm).

In greenhouse assays, plant height was significantly shorter in *M. bimucronata* seedlings grown with *S. virgata* seeds, regardless of the number of seeds (figure 2 a). The presence of *S. virgata* seeds negatively affected shoot diameter in *M. bimucronata* and *C. langsdorffii* seedlings (figure 2 b). Mean shoot dry weight of *M. bimucronata* seedlings was negatively and significantly affected by the presence of *S. virgata* seeds in greenhouse germination assays, whereas no effect was detected in *P. dubium* and *C. langsdorffii* seedlings (figure 2 c).

In the field assay, mean plant height of *C. langsdorffii* seedlings grown with five and 10 *S. virgata* seeds was significantly smaller (3.65 and 3.3 cm, respectively) (figure 3 a). Mean shoot diameter of seedlings grown in the field was not significantly affected by with the presence of *S. virgata* seeds (figure 3 b). The more gradual reduction in shoot dry weight was observed in *P. dubium* and *C. langsdorffii* seedlings grown with five and 10 *S. virgata* seeds (figure 3 c)

Plant height of *M. bimucronata*, *P. dubium*, and *C. langsdorffii* seedlings was significantly affected in greenhouse irrigation experiments (figure 4 a). Plant height of *M. bimucronata* and *P. dubium* seedlings treated with 0.5% *S. virgata* extract was significantly smaller compared to control seedlings, whereas 0.1 and 1.0% extracts had the strongest negative effect on plant height in *C. langsdorffii* seedlings (7.23 and 6.78 cm, respectively). In addition, mean shoot diameter of *P. dubium* and *C. langsdorffii* species in the greenhouse assay was significantly smaller in plants irrigated with either *S. virgata* seed extracts or catechin

Table 1. Germination rate (G%), germination speed index (GSI) and germination speed (GS) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seeds grown with *Sesbania virgata* (Cav.) Pers. seeds (0, 5, and 10 seeds).

Species	G%			GSI			GS		
	0	5	10	0	5	10	0	5	10
Laboratory									
Mimosa bimucronata	100a	100a	100a	0.45a	0.38ab	0.31b	2.15a	2.4b	2.6b
Peltophorum dubium	100a	100a	100a	0.32a	0.30a	0.31a	5.0a	5.1a	5.15a
Copaifera langsdorffii	85a	70a	65a	0.12a	0.06a	0.05a	13.20a	14.09a	13.75a
Greenhouse									
Mimosa bimucronata	100a	95a	85a	0.17a	0.15a	0.14a	7.25a	7.91ab	8.15b
Peltophorum dubium	100a	100a	85a	0.15a	0.14a	0.11b	8.3a	8.6ab	8.91b
Copaifera langsdorffii	90a	85a	80a	0.04a	0.03a	0.03a	14.07a	14.85a	14.42a
Field									
Mimosa bimucronata	60a	60a	75a	0.08a	0.08a	0.11a	5.3a	5.79a	5.24a
Peltophorum dubium	75a	40a	75a	0.08a	0.03a	0.08a	7.55a	6.81a	7.91a
Copaifera langsdorffii	75a	75a	75a	0.02a	0.02a	0.02a	18.72a	18.92a	17.84a

Means followed by the same letter within a row are not significantly different by the Tukey's test at $p < 0.05$.

Table 2. Germination rate (G%), germination speed index (GSI) and germination speed (GS) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seeds after treatment with *Sesbania virgata* (Cav.) Pers. seed coat extracts (0.1, 0.5, and 1.0% w/v) or catechin (C) solution. Control (0) corresponds to treatment with distilled water.

Species	G (%)					GSI					GS				
	0	0,1	0,5	1	C	0	0,1	0,5	1	C	0	0,1	0,5	1	C
Laboratory															
Mimosa bimucronata	100a	100a	100a	100a	95a	0.39a	0.31b	0.32b	0.26b	0.29b	2.15a	2.1a	2.3ab	2.6c	2.5bc
Peltophorum dubium	100a	100a	100a	100a	95a	0.22a	0.27a	0.23a	0.20a	0.19a	4.8a	5.05a	4.85a	4.95a	4.88a
Copaifera langsdorffii	100a	65a	95a	95a	80a	0.16a	0.04a	0.07a	0.07a	0.06a	12.9a	13.35a	13.3a	12.92a	13.05a
Greenhouse															
Mimosa bimucronata	100a	100a	80b	85b	100a	0.24a	0.22ab	0.17b	0.17b	0.17b	7.65a	7.9ab	8.25ab	8.08ab	8.5b
Peltophorum dubium	100a	100a	90a	100a	100a	0.16a	0.15a	0.15a	0.15a	0.14a	8.3a	8.3a	8.16a	8.0a	8.05a
Copaifera langsdorffii	90a	75a	90a	80a	80a	0.03a	0.0a3	0.04a	0.03a	0.03a	14.9a	15.7a	15.51a	14.87a	15.02a
Field															
Mimosa bimucronata	65a	40a	55a	50a	35a	0.08a	0.06a	0.08a	0.06a	0.05a	5.45a	4.0a	5.87a	4.27a	4.5a
Peltophorum dubium	60a	40a	85a	55a	80a	0.07a	0.04a	0.09a	0.06a	0.09a	7.61a	6.58a	7.93a	7.93a	7.83a
Copaifera langsdorffii	80a	75a	75a	80a	75a	0.02a	0.02a	0.02a	0.02a	0.02a	18.06a	18.31a	18.77a	18.15a	17.77a

Means followed by the same letter within a row are not significantly different by the Tukey's test at $p < 0.05$.

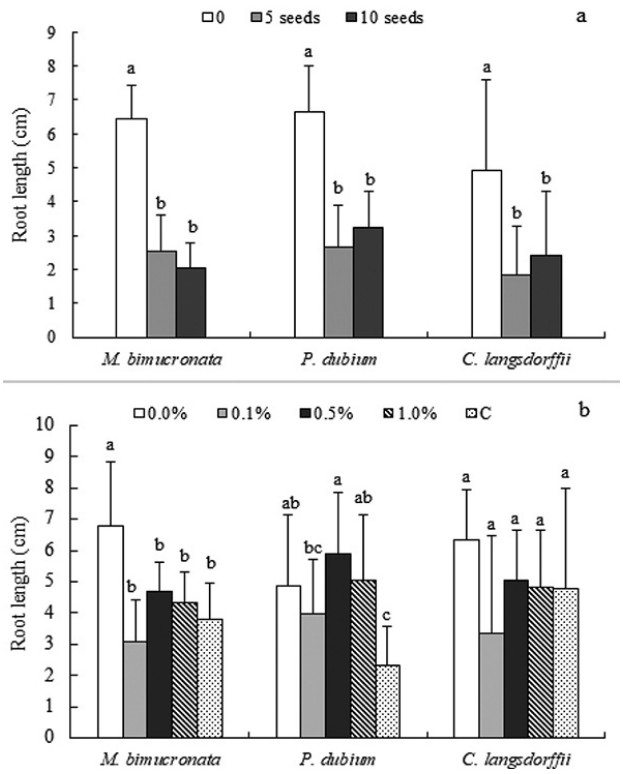

Figure 1. Mean root length (cm) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seedlings in laboratory assays. a. co-germination assays with 0, 5 and 10 seeds. b. irrigation assays with 0.0, 0.1, 0.5, 1.0% extracts, and catechin (C). Bars are means (±SD) of four replicates. Means followed by the same letter for each species and between treatments are not significantly different by the Tukey's test at $p < 0.05$.

(figure 4 b). For *M. bimucronata*, only 0.1% extracts did not induce reductive effects in relation to the control treatment. Mean shoot dry weight was significantly smaller in *P. dubium* and *C. langsdorffii* seedlings treated with *S. virgata* seed extracts, and the 0.5% extract had the strongest effect on *P. dubium* (figure 4 c).

In field assays, mean plant height and shoot diameter of plants treated with *S. virgata* seed extracts were significantly smaller only in *C. langsdorffii* plants and the 1.0% extract caused the most significant effect both for plant height (3.57 cm, figure 5 a) and shoot diameter (1.01 mm, figure 5 b). Mean shoot dry weight was significantly smaller only in *C. langsdorffii* with the greatest reductions observed in plants treated with 0.5 and 1.0% *S. virgata* seed extracts (0.11 and 0.09 g, respectively, figure 5 c).

Catechin quantification - The exudation profile of *S. virgata* seeds by HPLC during a 48 h imbibition period showed that peak catechin release was achieved at 18 h, followed by a gradual decrease in catechin concentration (figure 6).

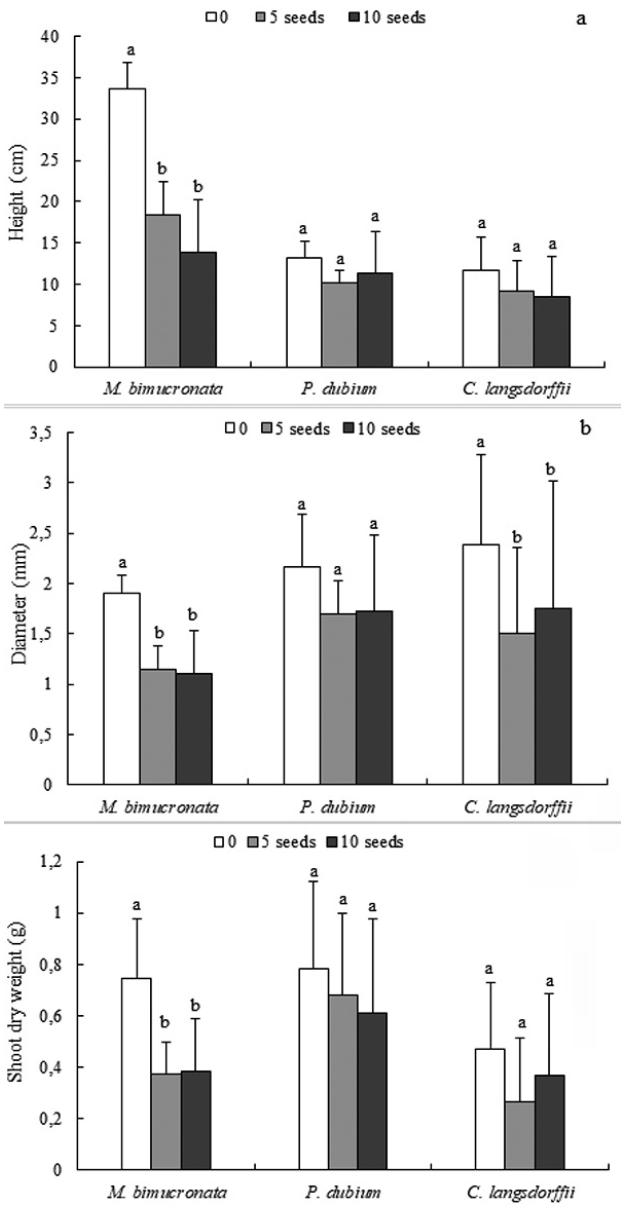

Figure 2. Mean plant height (cm), shoot diameter (mm) and shoot dry weight (g) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seedlings grown with *Sesbania virgata* (Cav.) Pers. seeds in greenhouse co-germination assays. a. plant height. b. shoot diameter. c. shoot dry weight. Bars are means (±SD) of four replicates. Means followed by the same letter for each species and between treatments (0, 5, and 10 seeds) are not significantly different by the Tukey' test at $p < 0.05$.

Discussion

Seeds germination - *Sesbania virgata* has been widely employed in restoration programs (Zanandrea *et al.* 2009, Florentino *et al.* 2009, Branzini *et al.* 2012). However, *S.*

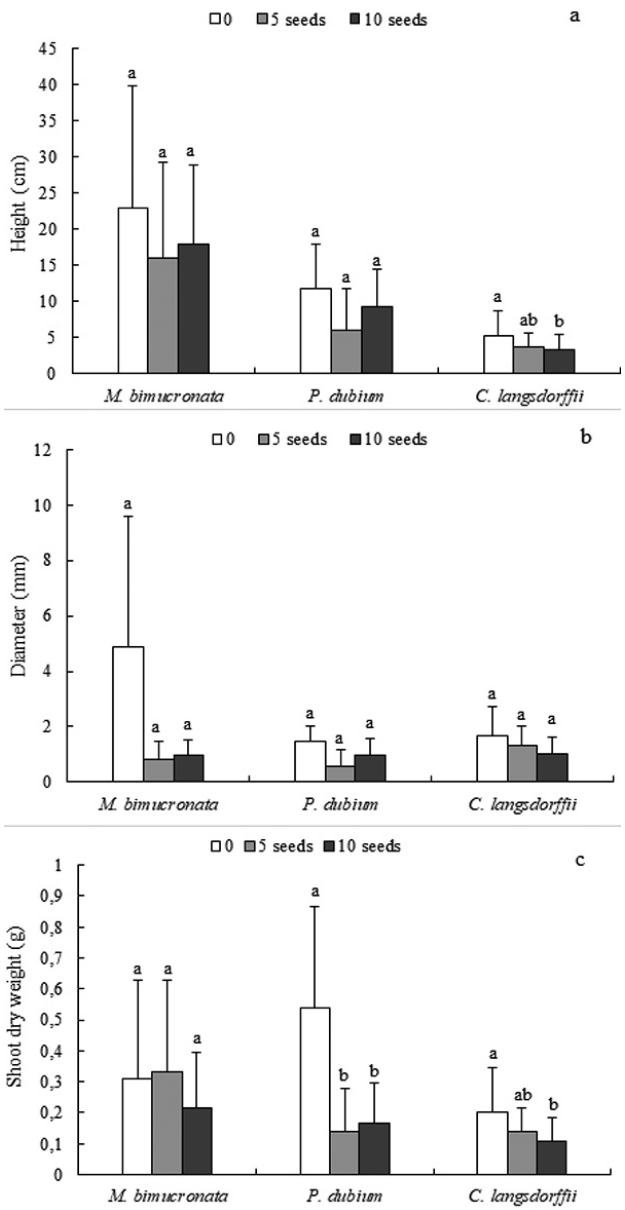

Figure 3. Mean plant height (cm), shoot diameter (mm) and shoot dry weight (g) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seedlings grown with *Sesbania virgata* (Cav.) Pers. seeds in field co-germination assays. a. plant height. b. shoot diameter. c. shoot dry weight. Bars are means (±SD) of four replicates. Means followed by the same letter for each species and between treatments (0, 5, and 10 seeds) are not significantly different by the Tukey's test at $p < 0.05$.

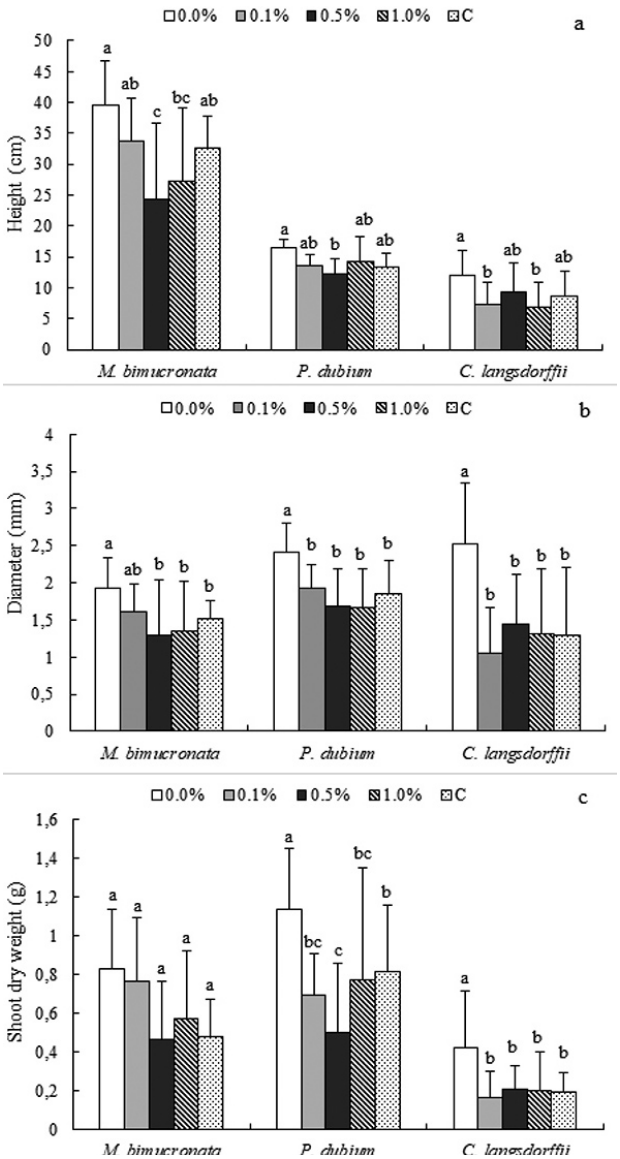

Figure 4. Mean plant height (cm), shoot diameter (mm) and shoot dry weight (g) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seedlings treated with *Sesbania virgata* (Cav.) Pers. seed extracts and catechin in greenhouse irrigation assays. a. plant height. b. shoot diameter. c. shoot dry weight. Bars are means (±SD) of four replicates. Means followed by the same letter for each species and between treatments (0.0, 0.1, 0.5, 1.0% extracts, and catechin [C]) are not significantly different by the Tukey's test at $p < 0.05$.

virgata is considered a superdominant species, which is able to outcompete other plant species by inhibiting their germination or development (Mignoni *et al.* 2017, El Id *et al.* 2015).

During seed imbibition, allelopathic compounds capable of inhibiting or retarding cell multiplication or growth may penetrate the seeds and delay germination (Bhadoria 2011). For instance, the presence of *S. virgata* seeds was shown to inhibit the germination of agronomic species (El Id *et al.*

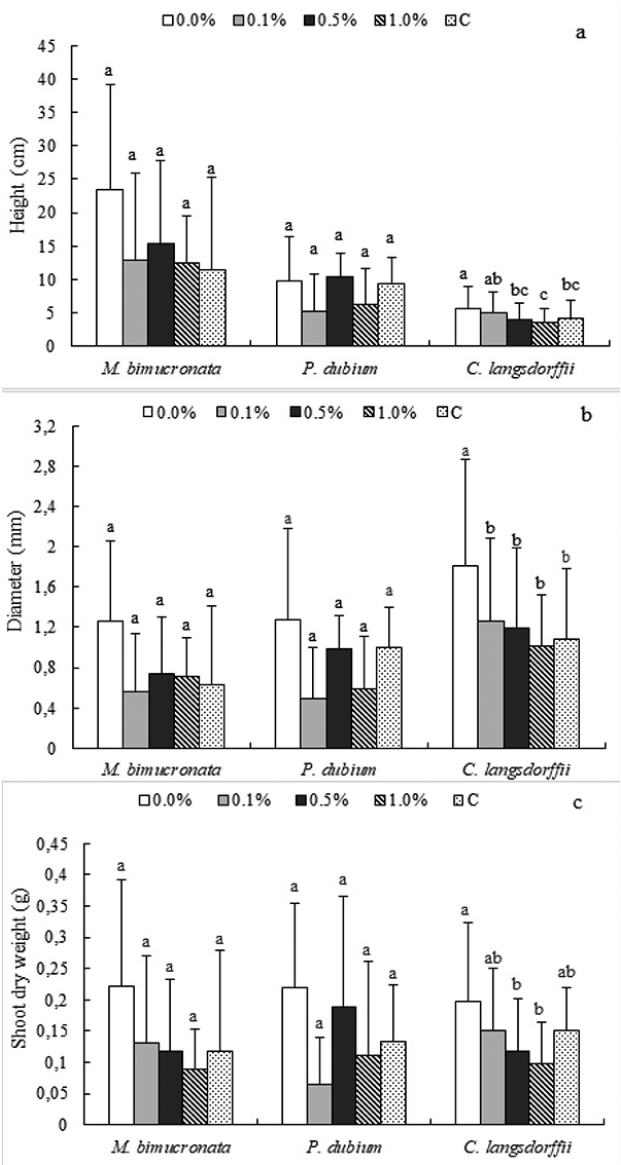

Figure 5. Mean (a) plant height (cm), (b) shoot diameter (mm) and (c) shoot dry weight (g) of *Mimosa bimucronata* (DC.) Kuntze, *Peltophorum dubium* (Spreng.) Taub. and *Copaifera langsdorffii* Desf. seedlings treated with *Sesbania virgata* (Cav.) Pers. seed extracts and catechin in field irrigation assays. a. plant height. b. shoot diameter. c. shoot dry weight. Bars are means (±SD) of four replicates. Means followed by the same letter for each species and between treatments (0.0, 0.1, 0.5, 1.0% extracts, and catechin [C]) are not significantly different by the Tukey's test at $p < 0.05$.

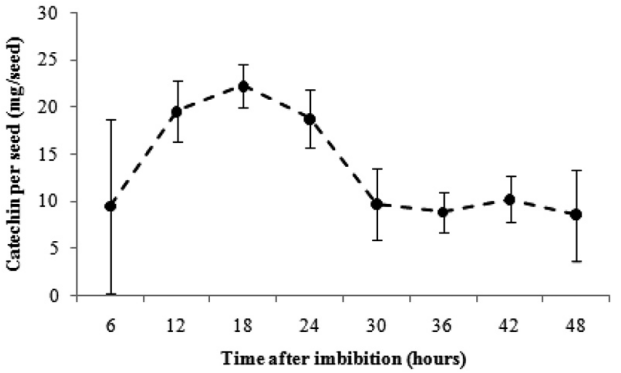

Figure 6. Catechin quantification (λ = 280 nm) in *Sesbania virgata* (Cav.) Pers. seed exudates by HPLC. Seeds were imbibed in distilled water and collected at six-hour intervals. Vertical bars represent the standard deviation of the mean (n = 3).

2015). Similarly, Mignoni *et al.* (2017) found that substances exuded by the seeds of *S. virgata* negatively affected the G% and GSI of *Leucaena leucocephala* (Lam.) de Wit. in germination experiments.

Even though *M. bimucronata* and *P. dubium* are pioneer species and may coexist with *S. virgata*, the lower GSI of *M. bimucronata* compared to that of *P. dubium* indicates that the former is more sensitive than the latter and the species studied by El Id *et al.* (2015) and Mignoni *et al.* (2017) to the phytochemicals released by *S. virgata*. A similar effect was observed in irrigation assays, in which *S. virgata* seed extracts were particularly effective against *M. bimucronata*, as indicated by the reduction in G% in laboratory test, by the increase in GS data in laboratory and greenhouse and GSI, in laboratory and greenhouse tests. The more significant effect of *S. virgata* on the GS and GSI of *M. bimucronata* and the lack of significant changes in the germination process of *P. dubium* and *C. langsdorffii* indicate that one or more stages of the germination process or mechanisms other than germination can be affected by compounds released from *S. virgata* seeds.

The lack of a significant effect on the GS and GSI of *M. bimucronata*, *P. dubium*, and *C. langsdorffii* under field conditions can be explained by the interference of environmental factors such as rainfall or microbial degradation in the soil on the action of allelopathic compounds (Trezzi *et al.* 2016). Moreover, forest species are not always sensitive to these substances. It should also be noted that plant resistance or tolerance to secondary metabolites is believed to be an intrinsic trait of the species mediated by environmental conditions (Inderjit & Duke 2003, Inderjit *et al.* 2011), which may explain the lack of negative effects on the germination of the species examined in this study.

Our results indicate that the phytochemicals released by *S. virgata* do not target seed germination in the species tested and provide the basis for future investigation of other species that use allelochemicals as part of their ecology. Future studies should target the mechanisms of action of the allelochemicals produced by *S. virgata* seeds, which provide the species with a competitive advantage in natural habitats.

Initial growth - Seedling growth rather than germination may be more sensitive to allelochemicals. According to Rice (1979), when seeds of sensitive species are exposed to allelochemicals, germination may be inhibited and their seedlings may also be subsequently affected. For instance, Mignoni *et al.* (2017) showed that *L. leucocephala* seedlings developed a shortened, darkened hypocotyl in seedling roots when grown with *S. virgata* seeds, indicating that compounds released by *S. virgata* seeds can inhibit the growth of different organs in some plant species. A similar effect was observed in the current study, as indicated by the reduction in root length, seedling height, shoot diameter, and shoot dry weight of plants grown with *S. virgata* seeds or irrigated with its extract.

Data for (+)-catechin release by *S. virgata* seeds are shown in figure 8. Peak (+)-catechin release was achieved up to 18 h after the onset of imbibition, confirming the findings of Simões *et al.* (2008). Interestingly, radicle protrusion in *M. bimucronata* occurs up to 24 h after the start of imbibition (data not shown). Because this species was one of the most affected in both laboratory and greenhouse assays, it is likely to have come into greater contact with compounds exuded by *S. virgata* compared to *P. dubium* and *C. langsdorffii*.

The initial development of *P. dubium* was less affected by compounds released by *S. virgata* seeds compared to that of *M. bimucronata* and *C. langsdorffii* in germination tests in the greenhouse and field. The weak effect of *S. virgata* compounds on *P. dubium* seeds may be related to the presence of galactomannan in the latter endosperm cell walls, which acts as a buffering physical barrier that controls the entry of water and other substances into the seed (Tonini *et al.* 2006, Buckeridge 2010). Thus, the galactomannan stored in the endosperm could also regulate the entry of allelochemicals released by *S. virgata*, preventing the initial contact of the *P. dubium* embryo with these substances in the first 24 h of imbibition.

After the degradation of galactomannan, the *P. dubium* embryo may have come into contact with the substances exuded by *S. virgata* seeds, possibly causing the adverse effects observed on root length and shoot dry weight. Thus, plant development was negatively affected in *P. dubium* seeds irrigated with *S. virgata* seed extracts, resulting in reduced seedling root length, height, shoot diameter, and shoot dry weight. In addition, this finding suggests that compounds other than catechin with allelopathic potential in the tegument of *S. virgata* seeds are not released during the first stages of imbibition, because plant development was more affected by application of seed extracts than germination with *S. virgata* seeds.

These allelochemicals may have been released later and been able to inhibit the development of *M. bimucronata* and *C. langsdorffii*. In fact, the alkaloid 'sesbanimida A' has high phytotoxic potential, is capable of inhibiting the growth of other plant species, and has been detected in *S. virgata* seeds (van Staden & Grobbelaar 1995, Simões *et al.* 2008). Additionally, Simões *et al.* (2008) reported the presence of the flavonoid 'quercetin' in the tegument of *S. virgata*. These compounds are toxic and may have adversely affected some parameters related to the development of *M. bimucronata* and *C. langsdorffii* seedlings.

The initial growth of *C. langsdorffii* seedlings was significantly reduced in nearly all treatments under nearly all conditions. The onset of *C. langsdorffii* germination occurred between days 18 and 21 in the laboratory and 26 and 30 in the greenhouse and field. In the field, this species was sensitive to commercial catechin, aqueous *S. virgata* extracts, and *S. virgata* seeds. This result suggests *S. virgata* seeds have compounds with inhibitory potential, which may have persisted in the environment during the bioassay period. According to Del Fabbro *et al.* (2014), allelopathic compounds can persist in soils. Phytochemicals can combine in the soil in various ways and may strongly affect the metabolism of other plants (Inderjit *et al.* 2011).

Catechin has allelopathic properties and is found in *S. virgata* seeds (Simões *et al.* 2008). The catechin molecule contains reactive hydroxyl groups, which can be oxidized by free radicals. Thus, flavonoids may act directly sequestering free radicals, resulting in a more stable chemical structure (Nijveldt *et al.* 2001). When catechins occur in a chemically uncontrolled condition, they may undergo changes that result in the loss of their inhibitory potential. Moreover, it has been argued that this molecule has a short half-life in the soil, rapidly degrading and losing its phytotoxicity (Inderjit *et al.* 2008, Duke *et al.* 2009). Thus, the intensity of catechin effects not only varies among species, but also depends on the experimental or other prevailing conditions (Buta & Lusby 1986, Weir *et al.* 2006). In the current study, the intensity of catechin effects was different in the field and the laboratory, because laboratory conditions are usually more stringently controlled and it is possible to eliminate external factors that may affect the experiment results.

The catechin molecule is known to be more unstable in soils with alkaline pH (Blair *et al.* 2005, Furubayashi *et al.* 2007) and to have greater toxicity in soils with higher organic matter content (Inderjit *et al.* 2008). Brazilian soils are mostly acidic and field trials in the current study were conducted near a forest fragment in an area with a large supply of organic matter. Thus, despite its short half-life in the soil, catechin may have had a negative effect on plant development, as shown by the significant reduction in growth parameters for plants irrigated with catechin extracts, especially in field assays. At the same concentration (1 mg mL^{-1}), the effect of irrigation with catechin was weaker compared to aqueous extracts from the tegument of *S. virgata* seeds. This result also suggests that, in addition to catechin, *S. virgata* seed extracts contain other compounds with inhibitory potential.

In conclusion, the lack of effect of seed extracts on the germination of native plant species suggests that the allelochemicals produced by *S. virgata* seeds act mainly during the post-germination stage rather than during seed germination. Seedling development may have been inhibited by the presence of catechin or other compounds in the tegument. It is possible that these compounds remain in the environment for some time, affecting the development of the species tested. In addition, *M. bimucronata* and *C. langsdorffii* seedlings were more sensitive to the allelochemicals released by *S. virgata* during their initial development than *P. dubium*. This result suggests that resistance to allelochemicals is not linked to the successional stage of a species but is determined by intrinsic characteristics that provide the capacity to tolerate the phytotoxins released by *S. virgata* seeds. The high competitive ability of *S. virgata* across habitat types against several plant species can be explained by the production of these phytotoxins, together with its high capacity for soil cover, seed bank formation, and affinity for modified soils.

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