

Phytotoxicity of mimosine and albizziine on seed germination and seedling growth of crops and weeds

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ABSTRACT

Mimosine and albizziine are non-protein amino acids produced by certain legume species that possess allelopathic activity. Although these allelochemicals have exhibited phytotoxicity in some bioassays, all test plants were not affected. We tested these compounds at various concentrations (10^{-3} to 10^{-5} M) for allelopathic effects on seed germination, growth, and greening of weeds and crops: hemp sesbania (*Sesbania exaltata*) and sicklepod (*Senna obtusifolia*) and wheat (*Triticum aestivum*). We also examined *in vitro* effects of these chemicals on the enzymatic activity of cysteine synthase from sicklepod tissue. Mimosine at 10^{-3} M reduced germination (by ~ 40%) in both wheat and sicklepod. The greatest growth inhibition occurred, when these compounds were supplied via seed imbibition, but some inhibition also was noted in root feeding and sprays of the chemicals on seedlings. Mimosine and albizziine inhibited chlorophyll development in hemp sesbania and sicklepod cotyledons at all concentrations tested. Chlorophyll inhibition ranged from 25 to 40 % for albizziine and from 20 to 97% for mimosine. Both compounds caused a small inhibition (10%) of sicklepod cysteine synthase activity. Generally mimosine was the more phytotoxic compound.

Key words: Allelochemical(s), cotyledon greening, inhibition, non-protein amino acid, root length, seed germination, seedling growth, shoot elongation, hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. Ex A.W. Hill], sicklepod (*Senna obtusifolia* L.), winter wheat (*Triticum aestivum* L.)

INTRODUCTION

Mimosine is a toxic, heterocyclic, non-protein amino acid found in the leaves, stems and seeds of *Mimosa pudica* L. and *Leucaena leucocephala* L. (16). It has insecticidal (7), fungicidal (10), and potentially herbicidal (8) properties. The toxicity of mimosine to animals is well documented in the literature. For example, large doses of mimosine can cause hair loss in goats (9,15), as well as other animals, and consumption of mimosine enlarged the goiters in calves of dairy cattle (16). The allelopathic nature of *L. leucocephala* and the allelochemical mimosine has been recently reviewed (8). Chou and Kuo (3) reported the weed exclusion beneath *Leucaena* canopy, while Prasad and Subhashini (13) reported mimosine inhibited seed germination and shoot

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length and nitrate reductase, peroxidase, catalase and IAA oxidase in rice (*Oryza sativa* L.). In bioassays mimosine inhibited lettuce seed germination, but had no effect on the growth of *Lemna minor* (16).

Albizzine is also a non-protein amino acid and is common component of many *Acacia* and *Albizzia* species, where it is a major component of the free amino acid fraction in the seed (16). Its structural analogy with glutamine suggests a potential for acting as a competitive antagonist of the protein amino acid. However, albizzine does not compete with glutamine for the active site of glutamyl-tRNA synthetase (16). It has insecticidal properties (12) and inhibits lettuce seed germination and *Lemna minor* growth (16).

Mimosine is a known pyridoxal phosphate inhibitor and pyridoxal phosphate can interact with many amino acids including glutamine (11). Furthermore, when supplied with certain substrates the pyridoxal-requiring enzyme, cysteine synthase can synthesize mimosine (17). Of the two compounds, albizzine has been less studied. For example, 160 citations for mimosine were found from the mid-1970s to the present, while only 17 citations were found for albizzine.¹

Our objectives were to examine and compare the effects of these two compounds on seed germination, seedling growth, cotyledon greening and on the activity of cysteine synthase from one of our test plant species. Two plant species tested were hemp sesbania and sicklepod which are very troublesome dicotyledenous weeds in the southern U.S. (18). Wheat was also used in some bioassays as a monocot test plant.

METHODS AND MATERIALS

Seed germination: Seed germination tests and seedling bioassays were performed as previously described (6). Twelve seeds of hemp sesbania [*Sesbania exaltata* (Ref.) Rydb. Ex A.W. Hill], sicklepod (*Senna obtusifolia* L.), and winter wheat (*Triticum aestivum* Lam.) were placed on filter paper moistened with sufficient solution to insure imbibition and germination. Mimosine and albizzine^{2,3} were tested at 10^{-5} M, 10^{-4} M and 10^{-3} M concentrations. Distilled water was used as control. The study was conducted in dark and germination (radicle protrusion) was determined at 24 h, 48 h and 72 h after imbibition. Shoot and radicle length were measured at 72 h. The experimental design was a 2 by 3 factorial with three replications per treatment. Data were subjected to ANOVA and means within a test species were separated using Duncan's multiple range tests ($P \leq 0.05$).⁴ Percentage data were transformed using an arc sin-square root transformation. However, since there was no difference between the statistical results using transformed or non-transformed data, the non-transformed data are presented.

Shoot elongation: Ten, four-day-old, dark-grown (25 °C) hemp sesbania and sicklepod seedlings grown in paper towel cylinders (6) were selected for uniformity. Seedlings were placed in fresh towel cylinders moistened with distilled water, the shoots were mist-sprayed with either mimosine (10^{-3} M), albizzine (10^{-3} M), or water (control) until fully wet, and placed in a dark chamber (25 °C). Shoots were measured at 72 h after treatment. Experimental design and statistical analysis were performed as described earlier.

¹ AGRICOLA database, U.S. Department of Agriculture, National Agricultural Library.

² SIGMA, St. Louis, MO, USA

³ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

⁴ SYSTAT, SSP Inc., Chicago, IL, USA

Root feeding: Ten, four-day-old, dark-grown (25 °C) hemp sesbania and sicklepod seedlings were placed in small test tubes containing either mimosine (10^{-3} M), albizziine (10^{-3} M), or water (control) and placed in a dark chamber (25 °C). Shoots were measured at 72 h after treatment. Experimental design and statistical analysis were performed as described earlier.

Cotyledon greening: Cotyledons were excised from four-day old, dark-grown (25 °C) hemp sesbania and sicklepod seedlings and imbibed in mimosine, albizziine or water (control) in dark for 2 h. After the dark period, the cotyledons were exposed to continuous, low intensity ($70 \mu\text{E m}^{-2} \text{sec}^{-1}$) light for 48 h. Chlorophyll was extracted with DMSO (5) and measured spectrophotometrically (1). Both compounds were tested at 10^{-5} M, 10^{-4} M and 10^{-3} M concentrations and results are expressed as percent of control. Six cotyledons were used per treatment and each treatment was replicated three times. Experimental design and statistical analysis were performed as described earlier.

Enzyme assay: Cysteine synthase (O-acetyl-L-serine sulfhydrylase) was extracted from plant tissues in 10mM potassium phosphate buffer, pH 8.0, containing dithiothreitol (20 mM). *In vitro* activity of cysteine synthase was determined spectrophotometrically, based on the formation of the ninhydrin-cysteine complex (4). Briefly the assay was performed in a total final volume of 1.0 ml containing 100 mM phosphate buffer, pH 7.8, 0.01 to 0.05 mg protein, 5 mM O-acetylserine, 1 mM Na_2S , 1 mM dithiothreitol, 0.025 mM pyridoxyl-5'-phosphate and mimosine or albizziine to attain a final concentration of 10^{-3} M. Assay mixtures were incubated at 37 °C for 10 min. when the reaction was terminated with 0.50 ml 20% trichloroacetic acid (w/v). Precipitated protein was removed by centrifugation (2000 x g for 10 min). A 1 ml aliquot of the supernatant was added to 1.5 ml ninhydrin reagent [250 mg ninhydrin in 20 ml glacial acetic acid: concentrated HCl (4:1 v/v)] and the mixture was heated in a boiling water bath for 6 min. and cooled. The absorbance was measured at A_{560} nm. Protein content in plant extracts was measured using the Bradford reagent (2).

RESULTS AND DISCUSSION

Seed germination was unaffected by mimosine or albizziine concentrations less than 10^{-3} M (data not shown). At 10^{-3} M concentration germination was slightly inhibited at 24 h by both compounds. However, at 48 h only wheat and sicklepod were significantly different from the controls and only in the mimosine 10^{-3} M treatment (Figure 1). At 48 h albizzine had no effect on germination, and hemp sesbania germination was not affected by either compound. By 72 h, there was no difference between the treatments and control for any species. Although delayed germination by an allelochemical is typically reported in literature, in most cases allelochemicals have more effect on radicle and/or shoot elongation (seedling growth) than on germination (15,19). Our study demonstrated that shoot and radicle elongation were significantly inhibited by 10^{-3} M mimosine and albizziine at 72 h. The shoot length of all species was inhibited by mimosine at 10^{-3} M, while only sicklepod and winter wheat were inhibited by albizziine at 10^{-3} M (Figure 2A).

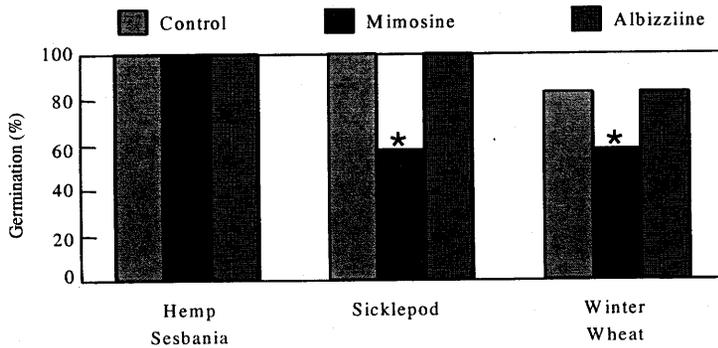


Figure 1. Effect of mimosine (10^{-3} M) and albizziine (10^{-3} M) on seed germination of hemp sesbania, sicklepod and winter wheat. Within a species, an asterisk indicates a mean which is significantly different from the control ($P \leq 0.05$).

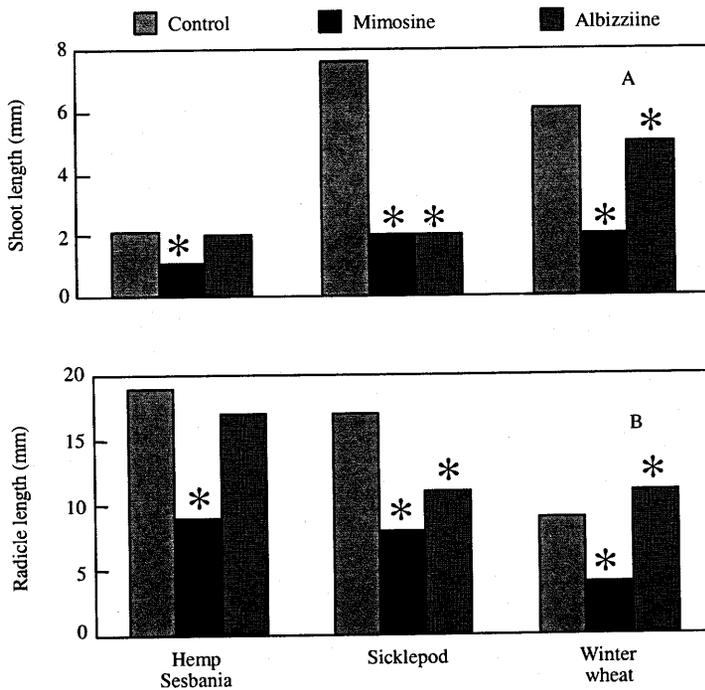


Figure 2. Effect of mimosine (10^{-3} M) and albizziine (10^{-3} M) on shoot (A) and radicle (B) elongation of hemp sesbania, sicklepod and winter wheat at 72 h. Within a species, an asterisk indicates a mean which is significantly different from the control ($P \leq 0.05$).

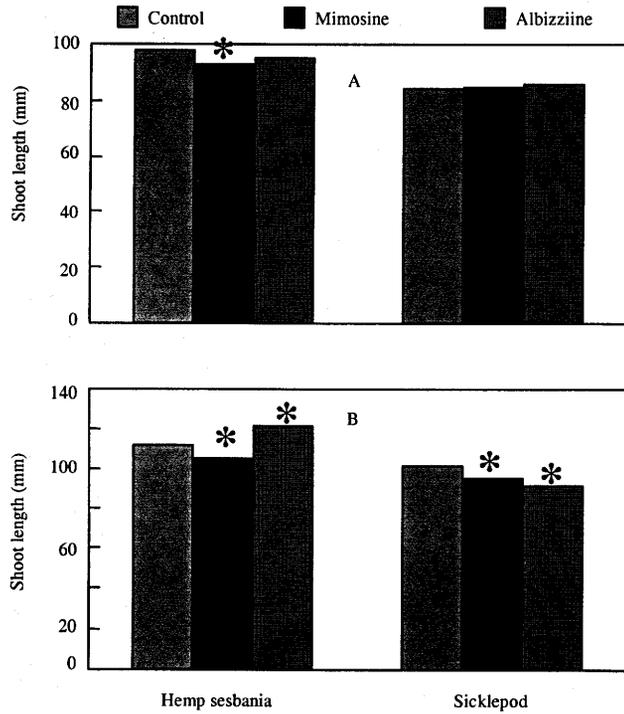


Figure 3. Effects of mimosine (10^{-3} M) and albizziine (10^{-3} M) on shoot elongation of dark grown hemp sesbania and sicklepod seedling applied as a spray (A) or root-fed (B). Within a species, an asterisk indicates a mean that is significantly different from the control ($P \leq 0.05$).

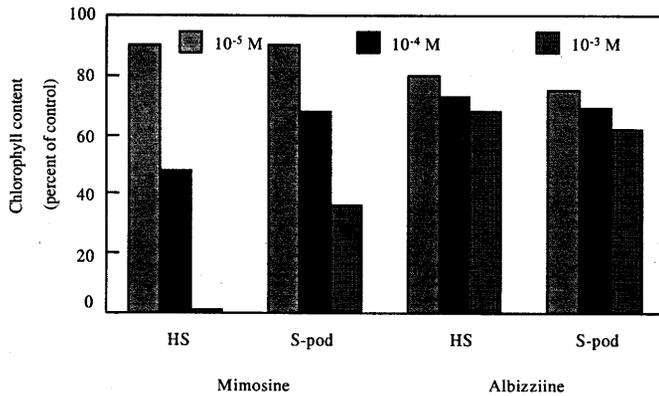


Figure 4. Effects of mimosine and albizziine on chlorophyll development in hemp sesbania (HS) and sicklepod (S-pod) cotyledons. All treatment means are significantly different from the controls at $P \leq 0.05$.

Similar results were obtained in the radicle length (Figure 2B). Overall only the 10^{-3} M concentration of either compound had a significant effect on germination and seedling growth. In addition, mimosine at 10^{-3} M proved more toxic in these bioassays than albizziine. Because only the largest concentration displayed significant effects, the 10^{-3} M concentration was used in the foliar and root-fed applications.

When the 10-day-old seedlings were sprayed with an application of 10^{-3} M mimosine, the shoot length of hemp sesbania was decreased, while the shoot length of sicklepod was slightly increased than control but the difference was not significant (Figure 3A). The application of 10^{-3} M albizziine did not affect the shoot length of either species. Essentially mimosine and albizzine sprayed on the test plants at 10^{-3} M concentration had no effect.

The shoot length of hemp sesbania was inhibited by mimosine, but was stimulated by albizziine at 10^{-3} M concentration, when the material was taken up by the roots (Figure 3B). However, both compound inhibited the shoot length in sicklepod, with the effect of albizzine was slightly greater than mimosine (Figure 3B).

Both compounds significantly inhibited the cotyledon greening (Figure 4). In hemp sesbania, albizziine at 10^{-5} M, 10^{-4} M and 10^{-3} M concentrations reduced the chlorophyll content by 20%, 27% and 32%, respectively, while the same concentrations reduced the sicklepod chlorophyll content by 25%, 32% and 38%, respectively. Albizzine appears to have the same effect on both species. Mimosine, had greater impact on cotyledon greening and hemp sesbania was more sensitive to this compound. Mimosine at 10^{-5} M, 10^{-4} M and 10^{-3} M concentrations reduced the chlorophyll content in hemp sesbania by 10%, 52% and 98%, respectively, while these concentrations reduced the sicklepod chlorophyll by 10%, 31%, and 64%, respectively.

In vitro cysteine synthase activity of the enzyme prepared from sicklepod seedling was inhibited by 10% with both mimosine and albizziine at 10^{-3} M, when these allelochemicals were added simultaneously to the enzyme with the enzyme substrates (data not shown). Pre-incubation of the enzyme prior to substrate addition, resulted in slightly higher inhibition (15%) in case of mimosine, but not for albizziine. This indicates that mimosine was more interactive than albizzine, presumably with pyridoxal phosphate. The effects of these compounds on extractable cysteine synthase would also be useful to examine.

CONCLUSIONS

John and Narwal (8) cited a study where an application of 1 mM (10^{-3} M) aqueous solution of mimosine adversely affected the germination and shoot and radical elongation of several species. However in the cited study, the sensitivity to mimosine varied by species, where rice was not inhibited and mung bean (*Vigna radiata* L.) was the most sensitive. In other work (13) rice seed germination was inhibited by 50 and 100 ppm (2.5×10^{-4} M and 5×10^{-4} M, respectively) concentrations of mimosine and shoot and root elongation was only significantly inhibited at 100 ppm concentration. In the present study the 10^{-3} M concentration, delayed germination of hemp sesbania, sicklepod and winter wheat at 48 h, and by 72 h the treatments were similar to the controls. Shoot length at 72 h after germination of all three test species was inhibited by 10^{-3} M application of mimosine,

while albizziine (10^{-3} M) only inhibited sicklepod and winter wheat shoot elongation. Radicle length was more inhibited by mimosine (10^{-3} M) than albizziine (10^{-3} M). A mist application of mimosine and albizziine (both at 10^{-3} M) did not show a significant biological effect on the shoot length of dark grown seedlings. However, root application of mimosine (10^{-3} M) inhibited the shoot elongation in dark grown seedlings of hemp sesbania and sicklepod, while the albizziine (10^{-3} M) stimulated shoot elongation of hemp sesbania and inhibited the elongation in sicklepod. Cotyledon greening was the most sensitive bioassay. Mimosine and albizziine at 10^{-5} to 10^{-3} M concentrations reduced the chlorophyll content of both hemp sesbania and sicklepod. However, mimosine appeared to be the most toxic compound in this bioassay. Throughout the study, mimosine displayed more phytotoxic ability than albizziine and as suggested in the literature concentrations of 10^{-3} M or greater are required to see significant delay in seed germination or inhibition of seedling growth. No other study has observed the effects of these two non-protein amino acids on cotyledon greening.

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