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# Germination of dormant seeds of *Stylosanthes humilis* as related to heavy metal ions

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## Abstract

Cadmium, copper and zinc ions at high concentrations partially released scarified freshly-harvested seeds of *Stylosanthes humilis* from physiological dormancy. This response to toxic metals increased along with seed postharvest ageing. Cobalt and silver ions, and abscisic acid impaired metal-promoted germination.

*Additional key words*: cadmium, cobalt, copper, dormancy break, silver, Townsville stylo seed, zinc.

## Introduction

The tropical legume Townsville stylo (*Stylosanthes humilis* H.B.K.) is an annual forage species whose seeds possess hard integuments; when harvested these seeds also exhibit physiological dormancy. The latter is gradually lost upon seed ageing (Burin *et al.* 1987, Vieira and Barros 1994). Treatment of seeds with high temperatures (Gardener 1975) or specific and unspecific regulators (Ballard and Buchwald 1971, Burin *et al.* 1987) breaks physiological dormancy. By supplying growth regulators in several combinations to dormant seeds, Burin *et al.* (1987) and Vieira and Barros (1994) concluded that at least two classes of growth substances, ethylene and cytokinin, were required for germination of Townsville stylo seeds. That ethylene is essencial for seed germination is shown by the following evidences: ACC, the immediate precursor of ethylene, partially breaks seed dormancy; seeds are also responsive to ethrel (2-chloroethylphosphonic acid), a well-known ethylene-releasing compound; inhibitors of ethylene biosynthesis, such as cobalt ions, and of ethylene action, such as silver ions, block germination of non-dormant seeds and of ACC-stimulated dormant seeds (Vieira and Barros 1994). Several plant systems respond to heavy metals by producing high amounts of ethylene; this stress response seems to be due to toxic effects of heavy metals at high concentrations (Matoo *et al.*

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*Abbreviations*: ABA- abscisic acid; ACC- 1-aminocyclopropane-1-carboxylic acid.

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1992). Metals were shown to retard dormancy break of rice seeds (Roberts 1963); in other instances, however, germination of dormant seeds was accelerated. In the latter case ethylene was likely to be involved (Gaal *et al.* 1988). Three approaches can be employed to test that hypothesis: *1*) determination of ethylene emanation; *2*) inhibition of ethylene biosynthesis; and *3*) blockage of ethylene action in metal-treated dormant seeds. In this work attempts were made to break physiological dormancy of Townsville stylo seeds with cadmium, copper and zinc ions. The involvement of ethylene in the process was investigated through inhibition of its biosynthesis and physiological action.

## Materials and methods

Townsville stylo plants were raised continuously in 3 kg plastic pots containing soil, sand and organic manure (2:1:1) in a greenhouse, in Viçosa, Minas Gerais State, Brazil. After harvested pods were kept under laboratory conditions; as a result of the many harvests and elapsed time, seeds of several postharvest ages were availabe for the assays. Seeds were freed from husks, scarified with fine sandpaper, sterilized with 0.5 % NaOCl for 10 min, and thoroughly washed with distilled water. They were then infiltrated by partial vacuum with chemicals dissolved in 10 mM Trizma-base buffer, pH 6.0, and containing 0.05 % Tween 80. Afterwards seeds were transferred to pre-heated Petri dishes (95 mm diameter), with a layer of *Whatman* paper *No. 1* at the bottom. Each dish received 15 $cm^3$ of test solution. Cobalt nitrate supplied $Co^{2+}$ and $Ag^{+}$ was provided as silver thiosulfate according to Reid *et al.* (1980). Cupric chloride was shown to be very toxic to the seeds and thus copper ions were provided as $CuSO_4$; $Cd^{2+}$ and $Zn^{2+}$ were supplied as $CdCl_2$ and $ZnCl_2$.

Germination tests were carried out in the dark at 30 °C; seeds with a protruded radicle about 3 mm long were considered as germinated. Daily germination counts were used to compose the index $\Sigma_5$, adapted from Timson (1965). This index simultaneously describes both percentage and speed of germination; thus, if all seeds germinated in the day 1 $\Sigma_5$ would be 500, if in day 2 400, and in day 6 it would be zero. The statistical design of the assays was based on a completely randomized distribution with five replicates of 50 seeds in a Petri dish, per treatment. Mean differences were detected by Scott-Knott's test (Scott and Knott 1974). Means followed by different letters (Table 1) were significant at the level of 5%.

## Results and discussion

A postgermination effect of $Cd^{2+}$ was observed in seedlings from seeds treated with the most concentrated solutions of this ion. Although germination course was normal, seedlings were stunted, and senesced and died in three weeks time. Meanwhile both germination and seedling growth were normal when seeds were treated with $Cu^{2+}$ and $Zn^{2+}$ at high concentrations.

Except for $Zn^{2+}$ whose maximal stimulation of seed germination occurred in the range $10^{-6}$ to $10^{-4}$ M, germination increased with increment of concentration of the other two ions (Fig. 1). Physiological dormancy of Townsville stylo seeds was partially broken also by ethrel, benzyladenine, the combination of these two regulators, ACC and thiourea (Burin *et al.* 1987, Vieira and Barros 1994). As was

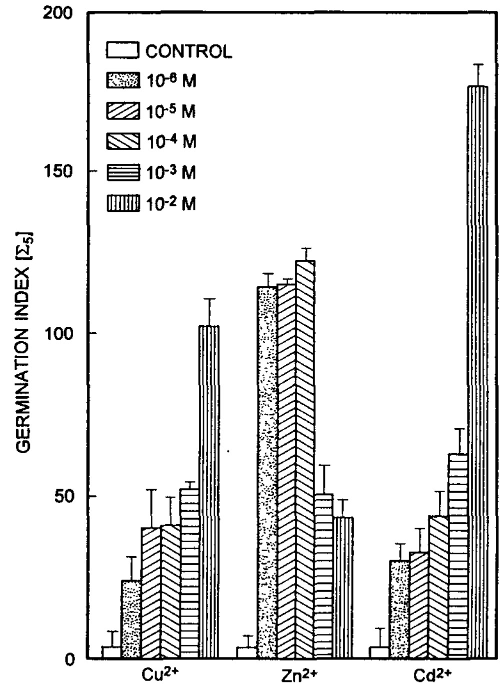


Fig. 1. Effects of $Cu^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ on the germination of dormant Townsville stylo seeds (36-d-old). *Bars* represent standard errors.

observed with all these regulators, seed response to heavy metals increased with their postharvest ages (Fig. 2). The increase in response was prominent in seeds up to an age about 60 d for $Cd^{2+}$ and 144 d for $Cu^{2+}$ and $Zn^{2+}$.

In bean leaves (Fuhrer 1982) and soybean cuttings (Pennazio and Roggero 1992) it was observed that $Cd^{2+}$ stimulated ethylene production. Incorporation of labelled methionine into ethylene by copper-treated tobacco leaf discs was shown to occur (Mattoo *et al.* 1992). Hence, the route methionine → ACC → ethylene was supposed to operate in these systems. Copper, which performs one electron oxireduction reactions may lead to the formation of free radicals. The latter may also be originated through mediation of lipoxygenase, such as in the case of zinc. Once formed free radicals may interfere with the conversion of ACC to ethylene; lipoxygenase may also be involved in this process (Vangronsveld *et al.* 1993).

Silver ions impair the expression of ethylene action (Reid *et al.* 1980), and inhibit germination of ethrel-stimulated dormant seeds of Townsville stylo (Vieira and Barros 1994). Likewise, $Ag^{+}$ substantially decreased ($Cu^{2+}$) or inhibited ($Cd^{2+}$ and $Zn^{2+}$) metal-promoted germination of seeds of the same species (Table 1), what means that metals elicited germination by triggerring ethylene production. It was likely that $Cu^{2+}$ and $Ag^{+}$ might be competing for the same site (Beyer *et al.* 1984) and hence inhibition of copper effect was incomplete. The possible route by which ethylene was originated was searched with the employment of modulators of ACC-synthase and ACCoxidase, key enzymes in the pathway leading to ethylene

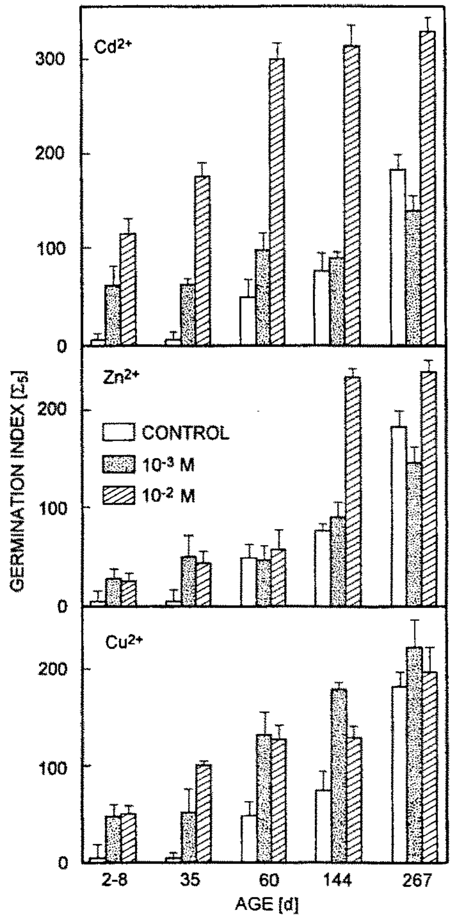


Fig. 2. Response to heavy metals of Townsville stylo seeds of several postharvest ages.

synthesis from methionine. In bean hypocotyl, $Co^{2+}$ inhibited ethylene production (Lau and Yang 1976); similarly, as shown in Table 1, these ions impaired the germination of metal-promoted seeds. In *Amaranthus caudatus*, ethylene production was decreased by ABA (Kepczynski 1986), possibly by decreasing ACC internal levels. Recently it has been suggested that, like $Co^{2+}$, the inhibitor also blocks ACCoxidase activity (Vieira and Barros 1994). Abscisic acid abolished the effects of the metals on seed germination (Table 1). In a series of assays (not shown), other blockers of ACCoxidase activity such as salicylic and acetylsalicylic acids (Leslie and Romani 1986), at $10^{-3}$ M, greatly reduced both $Cd^{2+}$-, $Cu^{2+}$- and $Zn^{2+}$-promoted germination of young seeds, and of untreated viable old seeds.

Table 1. Effects of $Ag^+$ ($10^{-2}$ M), $Co^{2+}$ ($2 \times 10^{-3}$ M) and ABA ($10^{-4}$ M) on germination (as expressed as $\Sigma_5$) of dormant Townsville stylo seeds (34 to 50-d-old) promoted by $Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$, each at $10^{-2}$ M.

| Treatment | $\Sigma_5$ | Treatment | $\Sigma_5$ | Treatment | $\Sigma_5$ | Treatment | $\Sigma_5$ |
|---|---|---|---|---|---|---|---|
| Control | 17.6 | | | | | | |
| $Cd^{2+}$ | 176.0 a | $Cd^{2+} + Ag^+$ | 12.8 d | $Cd^{2+} + Co^{2+}$ | 0.0 e | $Cd^{2+}$ + ABA | 2.0 e |
| $Zn^{2+}$ | 44.8 c | $Zn^{2+} + Ag^+$ | 0.0 e | $Zn^{2+} + Co^{2+}$ | 0.0 e | $Zn^{2+}$ + ABA | 2.0 e |
| $Cu^{2+}$ | 114.0 b | $Cu^{2+} + Ag^+$ | 52.0 c | $Cu^{2+} + Co^{2+}$ | 0.0 e | $Cu^{2+}$ + ABA | 1.6 e |

In bean roots and primary leaves $Cu^{2+}$ and $Zn^{2+}$ may elicit the appearance of certain isoperoxidases not stimulated by ethylene (Vangronsveld *et al.* 1993). Therefore, not always metal action is mediated through ethylene production solely. In any case, the results presented here indicated that heavy metals stimulated germination of Townsville stylo seeds through ethylene produced from methionine. The contention of Gaal *et al.* (1988) that ethylene mediates the effect of heavy metals on the break of seed dormancy was thus strongly supported.

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