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# Efficiency of selenium compounds in breaking dormancy of Townsville stylo seeds

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## Summary

Selenium compounds break dormancy of seeds of Townsville stylo (*Stylosanthes humilis*) through the induction of ethylene production. This was supported by the fact that antiethylenic substances inhibited germination of Se-treated dormant seeds. Under these conditions germination was restored by ethylene-producing compounds. Efficacy of the compounds was monitored through an Efficiency Index ($\mathbf{I_{ef}}$) which takes into account not only the maximal germination but also the optimal concentration responsible for eliciting the process. The most efficient compounds were $SeCl_4$, $SeO_2$ and $Na_2SeO_3$ and the least efficient were selenourea, $H_2SeO_4$ and selenomethionine. Although ethylene was required for dormancy breakage the compounds triggering the highest ethylene productions were not necessarily the same ones leading to the largest $\mathbf{I_{ef}}$(s). Hence it was concluded that the index is an overall integrating parameter, encompassing uptake of the compound, arrival to target cells and also post-ethylene biosynthesis effects.

## Introduction

Selenium (Se) is a micro-element essential to animal life, contributing four atoms to the molecule of glutathione peroxidase, a Se-protein with destroys $H_2O_2$ and hydroperoxides in the cytosol and mitochondrial matrix (Miller *et al*., 1991) and has also been associated with some anticarcinogenic properties. Its excess or lack in the soil where forage crops thrive may result in toxicity or deficiency leading to problems to the health of animals and humans (Welch, 1991). So far, however, no unequivocal essential function has been ascribed for Se in plant life, even though the element is amply metabolized by plants, mostly making usage of the metabolic routes of its analogue sulphur (Terry *et al*., 2000). In addition, specific pathways for Se have been identified in plants; for example Neuhierl and Böck (1996) purified a Se-cysteine methyltransferase from cultured cells of *Astragalus bisculatus*. Other specific pathways have been recently disclosed as a consequence of the development of studies on phytoremediation (Terry *et al*., 2000; Zhang *et al*., 2007).

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Because of the chemical similarity of the two elements, replacement of sulphur by Se in proteins and other compounds may occur leading to the toxicity displayed by Se.

Seeds of Townsville stylo (*Stylosanthes humilis* HBK), an annual tropical forage legume, have a relatively hard integument and also show a physiological dormancy which is gradually lost with increased postharvest age. After 12-15 months these seeds are completely germinable. Any condition or factor bringing about ethylene production by the seeds promotes dormancy breakage (Vieira and Barros, 1994; Pelacani *et al*., 2005). Recently ethylene was shown to be absolutely required for germination of Townsville stylo seeds (Ribeiro and Barros, 2006).

Selenomethionine (SeM) promotes dormancy breakage of Townsville stylo seeds and its effects are reverted by 2-aminoetoxyvinilglycine (AVG) and cobaltous ions, inhibitors of ethylene biosynthesis and by silver ions, inhibitors of ethylene action. This led Barros and Freitas (2000; 2001) to suggest that the SeM effects occur via ethylene production. Indications also exist that other Se compounds also break dormancy of Townsville seed. In the present work attempts were made to show that several other Se compounds structurally dissimilar to SeM also break dormancy of these seeds. Efficacy of the compounds in dormancy breakage was evaluated through an Efficiency Index ($\mathbf{I_{ef}}$). The relationships between dormancy breakage, ethylene production by seeds and $\mathbf{I_{ef}}$ were also investigated.

## Material and methods

*Plant material and general conditions*

Seeds were harvested from Townsville stylo plants cultivated in 3 $dm^3$ plastic pots in a greenhouse in Viçosa (30º 45' S, 42º 15' W), Minas Gerais state, Brazil. Seeds were kept dry under laboratory conditions until assays were conducted, when they were scarified with fine sand paper and sterilized with a 0.5% NaOCl solution for 10 minutes, and thoroughly washed with distilled water. Seeds were afterwards vacuum-infiltrated with test solutions and placed in either 9 cm petri dishes (10 $cm^3$ solution, 50 seeds, for germination assays) or sealed 25 $cm^3$ Erlenmeyer flasks (2 $cm^3$ solution, 25 seeds, for ethylene determination), both petri dishes and flasks with 2 Whatman Nº 1 filter paper layers at the bottoms. The petri dishes and Erlenmeyer flasks with filter paper were previously heated at 105ºC for 4 hours in an attempt to sterilize them. Germination was monitored in the dark at 30 ºC in a growth chamber (Forma Scientific Inc., Ohio, USA) for a period of 4-5 days. A seed was considered as germinated when the radicle had protruded about 3 mm. All test solution were prepared by dissolving chemicals (Sigma-Aldrich, USA) in water at pH 7.0 (HCl-KOH), containg 0.05% Tween 80.

*Ethylene quantification*

Air samples were taken from the atmosphere of the Erlenmeyer flasks in which seeds were placed (see above), using a gas tight syringe with its needle introduced through the rubber serum caps. Levels of ethylene were determined by the technique of Saltveit and Yang (1987). Air samples of 1 $cm^3$ were injected in a Hewlett-Packard 5890 series II chromatograph, equipped with a flame-ionization detector and a stainless steel column (1 m x 6 mm) packed with Porapak-N 80-100 mesh. Nitrogen carrier gas and hydrogen

flow rates were 30 $cm^3 min^{-1}$ and that of air 320 $cm^3 min^{-1}$. Column, injector and detector temperatures were kept at 60, 100 and 150 ºC, respectively. Ethylene peaks were recorded with an HP 3395 A integrator coupled to the chromatograph and measured by comparison with authentic ethylene standards.

After harvesting each air sample, flasks were unstoppered and their atmosphere exhausted in a fume-hood (no ethylene remained). Flasks were stoppered again and the process repeated until the end of the experiments.

*Germination inhibition and its reversion*

Germination inhibition of Se-treated seeds was accomplished in petri dishes with 0.1 mM AVG, 10 mM $Co^{2+}$ provided as $Co(NO_3)_2$, and 0.5 mM silver thiosulfate, the latter being freshly prepared before use (see Reid *et al*., 1980). Dormant seeds were supplied with combined solutions of Se compounds plus the inhibitors for 18 hours. After washing they were supplied with the inhibitor solution alone. In order to revert the action of ethylene inhibitors, after seeds had been exposed to Se compounds plus inhibitors, they were transferred to either a fresh solution of AVG plus 1.0 mM ACC, since AVG inhibits the activity of ACC synthase (Barbiker *et al*., 1994); $Co^{2+}$ plus 0.1 mM 2-chloroethylphosphonic acid (CEPA, an ethylene-releasing compound), since $Co^{2+}$ inhibits the activity of ACC oxidase (Gallardo *et al*., 1994); or to a 10 mM HCl pH 2.0 solution in an attempt to precipitate the silver ions likely coupled to a putative ethylene receptor (Knee, 1992). An account of these operations is shown in figure 1.

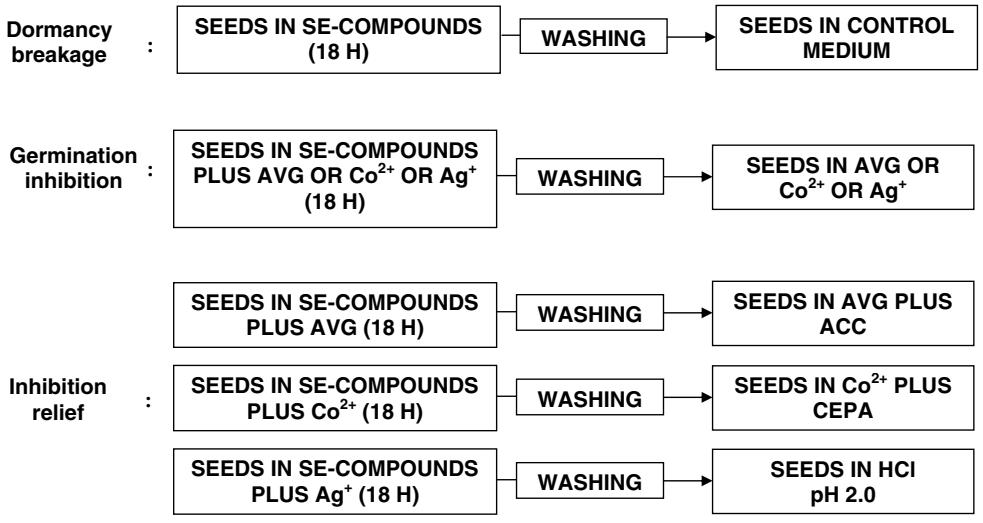


Figure 1. Flow diagram of assays conducted to break dormancy of seeds with Se compounds, to inhibit germination with antiethylenic substances simultaneously supplied with Se compounds, and to release inhibition with ethylene-producing compounds or with HCl. After permanence for 18 h in media shown in the left boxes, seeds were washed and then transferred to media presented in the right boxes where they were maintained until the end of assays.

*Time course of germination*
Several parameters of germination were determined for seeds imbibed in petri dishes. The germination curves were described by the Gompertz's function (Causton, 1983) with good precision. The intersecting point between the straight line projected from the ascending part of the integrative curve of germination and the abscissa axis defined the onset of the germination period. The moment of occurrence of the limiting point in the upper threshold sector of the curve indicated the germination saturation time, when germination was complete. The peak of the 1st derivative of the curve corresponding to the highest germination rate (inflexion of the integrative curve) occurred at the zero value of the 2nd derivative.

*Statistical layout*
All experiments were designed in a completely randomized distribution with replicates of five petri dishes with 50 seeds (for germination) or ten 25 $cm^3$ Erlenmeyer flasks with 25 seeds (for ethylene quantification). Germination percentage was transformed into arcsine $(\% \ G/100)^{0.5}$ to follow a normal distribution. Differences within each column in the tables for germination means were examined by the test of Scott and Knott (1974), and differences for means of ethylene amounts by the Tukey test. In all cases, they were tested at a significance level of $P \leq 0.05$.

## Results

*Establishment of working conditions*
At low to moderate concentrations Se compounds are toxic and hence several assays were carried out to determine their most effective levels (optimal concentrations) to break seed dormancy and the maximal time period for which seeds should be exposed to Se without showing toxicity symptoms. A 18 h exposure time under the optimal concentrations shown in table 1 proved to be the best ones and both (Se concentrations and exposure time) were used throughout. After exposure, seeds were then thoroughly washed and transferred to the control medium (water pH 7.0) or to another treatment medium (figure 1). At the end of main experiments (5th day), germinating media were replaced by thiourea 0.1 M to test for seed viability (Ballard and Buchwald, 1971); in every case the resulting germination was above 90%.

*Dormancy breakage and the Efficiency Index ($I_{ef}$)*
As seen in table 1, which presents averaged results of six assays, all soluble Se compounds tested broke dormancy of Townsville stylo seeds to different degrees. In terms of germination percentage the compounds displaying the greatest efficacy were selenourea (SeU) and $H_2SeO_3$, and the least effective one was SeM (and see also figure 1). The situation might be rather different if the results were expressed as the optimal concentration of the compounds which elicits the maximal germination, i. e., through an index describing the efficiency of the compound:

$$\mathbf{I_{ef} = C^{-1}. \frac{(G_x - G_o)\%}{100}}$$

in which **C** represents the optimal concentration, $\mathbf{G_x}$ the germination percentage promoted by the compound, and $\mathbf{G_0}$ the germination observed in the control seeds. The Efficiency Index ($\mathbf{I_{ef}}$) describes how much a unit germination is affected by a unit concentration, working thus by algorithms. By using this index and not the germination percentage by itself, the most efficient compounds in breaking seed dormancy were $SeCl_4$, $SeO_2$ and $Na_2SeO_3$ (table 2).

Table 1. Germination of dormant Townsville stylo seeds as affected by Se compounds at the optimal concentration tested (bold) and an exposure time of 18 hours. Afterwards seeds were transferred to water. Average of six experiments. Bars represent SE.

| Compound - Optimal concentration | Germination (%) in different assays | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± SE |
| Sodium selenate ($Na_2SeO_4$) - **2.0 mM** | 83.2 | 85.2 | 66.8 | 73.2 | 77.2 | 87.2 | **78.8 ± 3.2** a |
| Sodium selenite ($Na_2SeO_3$) - **0.2 mM** | 81.2 | 73.6 | 49.2 | 64.4 | 79.2 | 77.2 | **70.9 ± 5.0** b |
| Selenic acid ($H_2SeO_4$) - **1.0 mM** | 86.6 | 76.0 | 68.0 | 80.0 | 76.8 | 88.0 | **79.2 ± 3.0** a |
| Selenious acid ($H_2SeO_3$) - **0.6 mM** | 86.0 | 78.4 | 70.8 | 87.6 | 79.2 | 86.8 | **81.5 ± 2.7** a |
| Selenourea (SeU) ($CH_4N_2Se$) - **1.0 mM** | 95.2 | 87.2 | 61.6 | 90.8 | 87.2 | 89.6 | **85.3 ± 4.9** a |
| Selenomethionine (SeM) ($C_5H_{11}NO_2Se$) - **1.0 mM** | 56.8 | 56.4 | 55.6 | 60.4 | 70.4 | 74.8 | **62.4 ± 3.3** b |
| Selenium tetrachloride ($SeCl_4$) - **0.2 mM** | 86.4 | 79.4 | 47.6 | 78.4 | 80.8 | 78.0 | **75.1 ± 5.6** b |
| Selenium dioxide ($SeO_2$) - **0.2 mM** | 88.4 | 68.0 | 49.2 | 72.0 | 79.6 | 84.4 | **73.6 ± 5.8** b |
| Control (dormant) | 1.6 | 2.0 | 14.0 | 7.6 | 9.2 | 11.6 | **7.7 ± 2.1** c |

Table 2. Efficacy of Se compounds in breaking dormancy of Townsville stylo as expressed as the Efficiency Index ($\mathbf{I_{ef}}$) calculated as described in the text, employing the germination percentages presented in table 1.

| Compound | $\mathbf{I_{ef}}$ in different assays | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± SE |
| $Na_2SeO_4$ | 0.41 | 0.42 | 0.26 | 0.33 | 0.34 | 0.38 | **0.36 ± 0.02** d |
| $Na_2SeO_3$ | 3.98 | 3.58 | 1.76 | 2.84 | 3.50 | 3.28 | **3.16 ± 0.32** a |
| $H_2SeO_4$ | 0.85 | 0.74 | 0.54 | 0.72 | 0.68 | 0.76 | **0.72 ± 0.04** c |
| $H_2SeO_3$ | 1.40 | 1.27 | 0.94 | 1.33 | 1.17 | 1.25 | **1.23 ± 0.07** b |
| SeU | 0.94 | 0.86 | 0.48 | 0.83 | 0.78 | 0.78 | **0.78 ± 0.06** c |
| SeM | 0.55 | 0.54 | 0.41 | 0.53 | 0.61 | 0.63 | **0.55 ± 0.03** d |
| $SeCl_4$ | 4.24 | 3.87 | 1.68 | 3.54 | 3.58 | 3.32 | **3.37 ± 0.36** a |
| $SeO_2$ | 4.34 | 3.30 | 1.76 | 3.22 | 3.52 | 3.64 | **3.30 ± 0.35** a |

*Dynamics of germination*

The dynamics of germination in petri dishes of Se-treated dormant seeds and untreated dormant and non-dormant seeds fitted to the Gompertz's function with good precision. Data derived from the curves shown in figure 2 were employed to construct table 3. It is seen that germination of Se-treated seeds lagged behind germination of non-dormant seeds; the onset of germination in the former group occurred much later than in non-dormant seeds, with the whole germination process taking much longer to complete (duration). However when the reference timing point was reset at the onset of germination instead of being kept at the onset of incubation, all the differences between the phase events of germination of non-dormant and Se-treated seeds were greatly attenuated or even disappeared. For instance, whilst the germination process was completed in about 12 hours for control non-dormant seeds it took 10, 13 and 20 hours for SeM-, $Na_2SeO_3$- and $H_2SeO_4$-treated seeds respectively. For those seeds the moment of occurrence of the maximal rates of germination was 1.2, 4.0, 3.4 and 3.0 hours after the start of germination.

Table 3. Some components of the germination dynamics (germination onset, maximal rate of germination, time taken for occurrence of maximal germination and duration of the germination process) derived from the Gompertz's curves of figure 2.

| Compound | From incubation | | | | From germination | |
|---|---|---|---|---|---|---|
| | Germin. onset (h) | Max. rate (seed $h^{-1}$) | Time max. germ. (h) | Duration (h) | Time max. germ. (h) | Duration (h) |
| $Na_2SeO_4$ | 18.2 | 9 | 22 | 36 | 3.8 | 17.8 |
| $Na_2SeO_3$ | 14.6 | 9 | 18 | 28 | 3.4 | 13.4 |
| $H_2SeO_4$ | 16.0 | 7 | 19 | 36 | 3.0 | 20.0 |
| $H_2SeO_3$ | 16.2 | 7 | 21 | 36 | 4.8 | 19.8 |
| SeU | 11.9 | 13 | 16 | 26 | 4.1 | 14.1 |
| SeM | 16.0 | 3 | 20 | 26 | 4.0 | 10.0 |
| $SeCl_4$ | 11.9 | 7 | 16 | 26 | 4.1 | 14.1 |
| $SeO_2$ | 12.4 | 7 | 17 | 32 | 4.6 | 19.6 |
| Control (dormant) | 17.2 | 1 | 22 | 36 | 4.8 | 18.8 |
| Control (non-dormant) | 5.8 | 10 | 7 | 18 | 1.2 | 12.2 |

*Relationships with ethylene*

On the grounds that Townsville stylo seeds require ethylene for dormancy-breakage and germination (Ribeiro and Barros, 2006), ethylene emanation was monitored in Se-treated and untreated seeds. In all cases the ethylene produced in sealed Erlenmeyer flasks containing Se-treated seeds (and untreated non-dormant seeds as well) surpassed by far the amounts produced by untreated dormant seeds (table 4). Therefore this Se-driven ethylene production by dormant seeds was likely to have led to their dormancy-breakage. The inhibitor of ACC oxidase, $Co^{2+}$, was highly effective in inhibiting germination of Se-stimulated seeds (table 5) followed by AVG, inhibitor of ACC synthase, and Ag+, inhibitor of ethylene response. In some cases inhibition was almost complete. Following the 18 hours exposure of seeds to Se plus AVG, Se plus $Co^{2+}$ and Se plus $Ag^{+}$ seeds were transferred respectively to a mixture solution of $Co^{2+}$ plus CEPA (to circumvent the blockage of ACC oxidase); AVG plus ACC (to overcome the blockage of ACC synthase); and to a 10 mM HCl solution pH 2.0. In all cases germination was restored to levels approaching or greater than 90%.

Table 4. Germination percentage and total ethylene produced by dormant seeds contained in 25 $cm^3$ Erlenmeyer flasks. Mean ± SE of 10 replicates. Germination was compared by Scott-Knott and ethylene by Tukey test at 5% level.

| Compound | Germination (%) | Ethylene (nmol g-1, FM) |
|---|---|---|
| $Na_2SeO_4$ | 74.0 + 3.1 d | 22.3 + 1,4 b |
| $Na_2SeO_3$ | 82.2 + 2.5 c | 19.4 + 1.1 b |
| $H_2SeO_4$ | 75.8 + 2.2 d | 23.4 + 1.9 b |
| $H_2SeO_3$ | 73.5 + 1.8 d | 15.9 + 0.8 c |
| SeU | 92.1 + 1.6 b | 27.8 + 1.3 a |
| SeM | 73.2 + 3.8 d | 31.3 + 1.9 a |
| $SeCl_4$ | 87.0 + 2.3 b | 22.3 + 0.8 b |
| $SeO_2$ | 81.3 + 3.2 c | 20.4 + 1.3 b |
| Control (dormant) | 15.7 + 2.3 e | 7.2 + 0.8 d |
| Control (non-dormant) | 98.5 + 0.6 a | 30.1 + 1.3 a |

Table 5. Inhibition of germination of Se-stimulated dormant seeds of Townsville stylo (left). Seeds were treated with Se compounds plus ethylene inhibitors ($Co^{2+}$, AVG and $Ag^+$) for 18 hours being afterwards (→) transferred to solution of the inhibitors only. Control dormant seeds were left in water. In order to revert the inhibition (below) seeds were treated with Se compounds plus the inhibitors for 18 hours, being afterwards (→) transferred to solutions of $Co^{2+}$ plus CEPA, AVG plus ACC and to an HCl solution. Control seeds were left in water being transferred then to the second media as described (see also figure 1). Comparisons were made in each column by Scott-Knott at 5% level.

| | Inhibition | | |
|---|---|---|---|
| Compound | Se + $Co^{2+}$ → $Co^{2+}$ | Se + AVG → AVG | Se + $Ag^+$ → $Ag^+$ |
| $Na_2SeO_4$ | 26.0 ± 3.3 a | 40.4 ± 2.8 a | 58.0 ± 3.4 a |
| $Na_2SeO_3$ | 12.8 ± 2.2 b | 27.6 ± 4.0 b | 34.8 ± 1.5 b |
| $H_2SeO_4$ | 16.0 ± 2.3 b | 41.2 ± 1.4 a | 56.8 ± 3.2 a |
| $H_2SeO_3$ | 13.6 ± 1.9 b | 33.2 ± 4.5 b | 61.6 ± 4.6 a |
| SeU | 12.0 ± 0.6 b | 11.6 ± 3.0 c | 9.6 ± 2.3 d |
| SeM | 0.4 ± 0.4 d | 0.8 ± 0.5 d | 23.2 ± 2.8 c |
| $SeCl_4$ | 7.6 ± 1.5 c | 31.6 ± 3.5 b | 37.2 ± 1.4 b |
| $SeO_2$ | 8.8 ± 1.5 c | 31.6 ± 2.8 b | 33.2 ± 3.9 b |
| Control | 0.0 d | 0.4 ± 0.4 d | 2.0 ± 0.9 e |

| | Reversion of inhibition | | |
|---|---|---|---|
| Compound | Se + $Co^{2+}$ → $Co^{2+}$ + CEPA | Se + AVG → AVG + ACC | Se + $Ag^+$ → HCl |
| $Na_2SeO_4$ | 95.2 ± 1.0 b | 84.8 ± 2.3 a | 88.8 ± 1.0 a |
| $Na_2SeO_3$ | 98.4 ± 0.4 a | 90.0 ± 1.9 a | 93.2 ± 1.4 a |
| $H_2SeO_4$ | 96.0 ± 1.4 a | 90.8 ± 3.1 a | 89.2 ± 1.7 b |
| $H_2SeO_3$ | 90.0 ± 2.5 b | 91.6 ± 3.3 a | 88.8 ± 2.6 b |
| SeU | 98.4 ± 1.2 a | 92.8 ± 3.3 a | 96.0 ± 1.1 a |
| SeM | 97.2 ± 0.8 a | 89.6 ± 1.2 a | 93.2 ± 2.1 a |
| $SeCl_4$ | 97.2 ± 1.0 a | 88.4 ± 3.3 a | 92.0 ± 2.0 a |
| $SeO_2$ | 98.0 ± 0.6 a | 90.0 ± 1.9 a | 94.4 ± 1.6 a |
| Control | 97.6 ± 0.8 a | 93.6 ± 2.3 a | 86.6 ± 1.2 b |

## Discussion

SeM has been described as a dormancy breaking agent for Townsville stylo seeds (Barros and Freitas, 2000; 2001). Herein it was shown that several other Se compounds whose structures do not resemble SeM at all may work in a similar way, and thus the list of Se compounds displaying similar effects has been extended (table 1, figure 2). The chemical species breaking dormancy of Townsville stylo seeds were highly diverse and the only chemical characteristic they share is the possession of a Se atom. Hence Se by itself seemed to constitute the dormancy-breaking agent.

Contrary to the effects in Townsville stylo seeds, $Na_2SeO_3$ and $Na_2SeO_4$ did not break dormancy of seeds of lettuce (Carlson *et al*., 1989), possibly because they have different requirements for dormancy breakage. However, Se promoted a beneficial effect on germination of *Vigna radiata* seeds (Lalitha and Easwari, 1995) and, at low concentrations, favoured the germination of seeds of *Trigonella foenum-graecum* by increasing the activity of hydrolytic enzymes (Sreekala and Lalitha, 1998). In bitter gourd (*Momordica charantia*) maintained under suboptimal temperatures, imbibition of seeds with $Na_2SeO_3$, restored their germination power (Chen and Sung, 2001), this likely being associated to the effect of Se compounds as antioxidant agents (Kostyshim *et al*., 1997).

When the compounds employed are grouped on the basis of their Se valence it is seen that, in the most efficient compounds for breaking seed dormancy, Se atoms display an oxidation state +4. It seems that the Se chemical valences +6 ($Na_2SeO_4$, $H_2SeO_4$), +2 (SeU) and -2 (SeM) confer a low activity to the compounds. Besides the difference in Se oxidation state between $SeO_3^{2-}$ and $SeO_4^{2-}$, it also seems that the more reduced form is much more rapidly incorporated into organic compounds, whereas $SeO_4^{2-}$ mostly accumulates into tissues (Souza *et al.*, 1998). If to work as a dormancy-breaking agent Se has to be incorporated into proteins (see below), this helps in explaining, at least partially, why $H_2SeO_3$ and $Na_2SeO_3$ displayed a much higher $\mathbf{I_{ef}}$ than $H_2SeO_4$ and $Na_2SeO_4$ (table 2).

All compounds listed hitherto possess a single Se atom in their molecules. In order to test whether two Se atom-containing molecules displayed a higher efficacy in breaking dormancy, dormant seeds were treated with five other different compounds (detailed data not presented). Results obtained were: diselenium dichloride ($Se_2Cl_2$) - $\mathbf{I_{ef}}$ 1.07, selenium oxychloride ($SeOCl_2$) - $\mathbf{I_{ef}}$ 1.03, selenocystine ($C_6H_{12}N_2O_4Se_2$) - $\mathbf{I_{ef}}$ 0.83, selenoethionine ($C_6H_{13}NO_2Se$) - $\mathbf{I_{ef}}$ 0.79 and selenopurine ($C_5H_4N_4Se$) - $\mathbf{I_{ef}}$ 0.47. It thus seems that notwithstanding the number of Se atoms there existing in the compound the molecule by itself acts as an integral entity in eliciting seed dormancy breakage.

In addition to comparing the effectiveness of different compounds in eliciting germination at their optimal concentrations, employment of $\mathbf{I_{ef}}$ offers some other advantages. Firstly, it deals with the net germination $\Delta\mathbf{G}$ (= $G_x$ - $G_o$) promoted by the compound and not only with total germination ($\mathbf{G_x}$). Thus, it can monitor the changes in dormancy degree because once the compound is no longer required for germination $\Delta\mathbf{G}$ becomes zero. Secondly, it can be used to determine the efficiency of inhibitory substances in which case $\Delta\mathbf{G}$ turns to be negative: the more negative $\Delta\mathbf{G}$ the stronger their inhibiting effect. Thirdly, it can compare chemicals of the more diverse kinds. On a

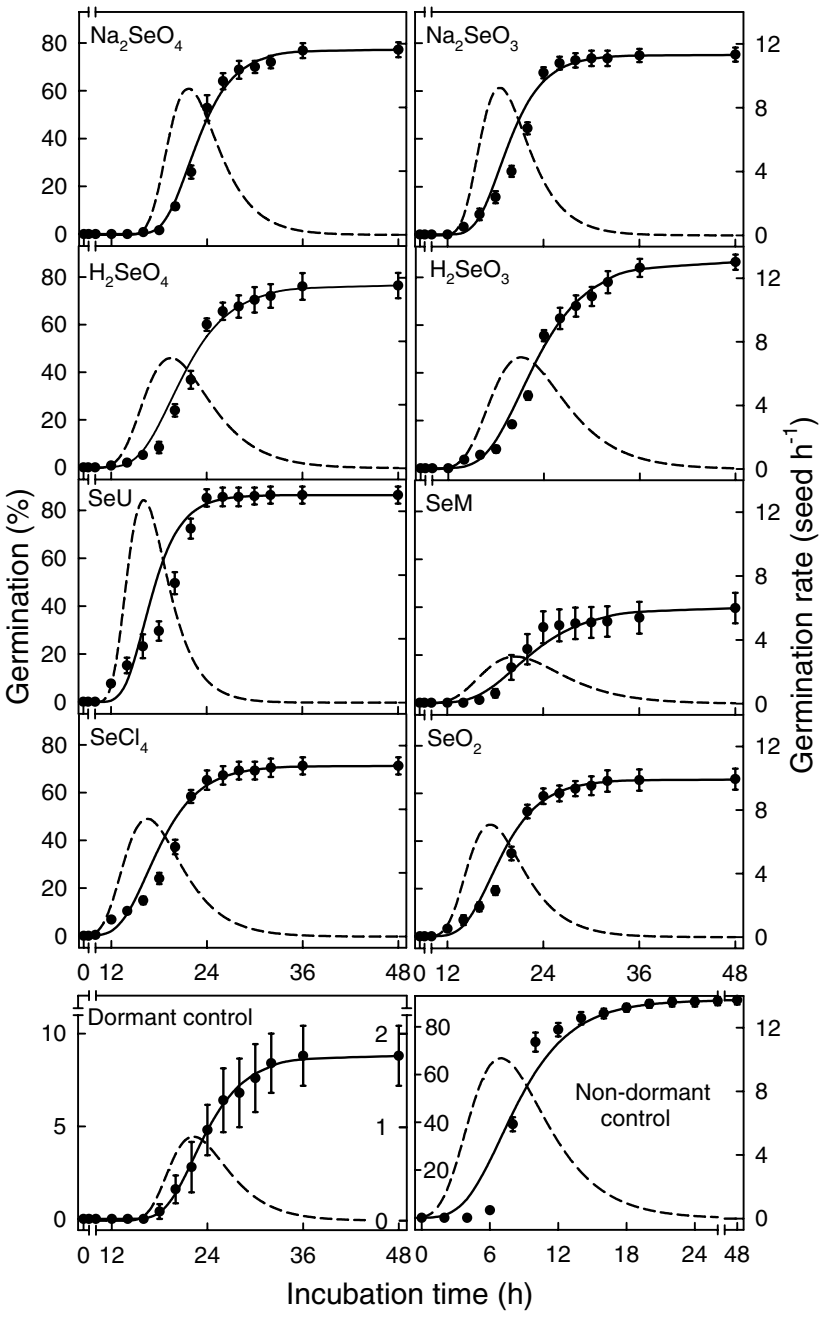


Figure 2. Germination course of dormant Townsville stylo seeds as treated with Se compounds at the concentrations shown in table 1. Note the different scales in the ordinate axis for the control dormant seeds and in the abscissa axis for the control non-dormant seeds. Bars represent SE.

mM basis, for instance, $SeCl_4$ produced an $\mathbf{I_{ef}}$ = 3.37 (table 2); CEPA (Vieira and Barros, 1994) an $\mathbf{I_{ef}}$ = 8.5, and the ethylene gas (Ribeiro and Barros, 2006) an $\mathbf{I_{ef}}$ = 170, showing the increasing $\mathbf{I_{ef}}$ displayed by a Se compound eliciting ethylene production (see below), by an ethylene-releasing compound and by the regulator itself.

Of the several advantages of usage of $\mathbf{I_{ef}}$, the greatest one is its simplicity. Once $\mathbf{I_{ef}}$ and the concentration of the compound eliciting dormancy breakage were known, the net germination ($\Delta\mathbf{G}$) is rapidly found. In other words provided that two parameters of the equation be at hand, the other one is easily estimated.

When the germination parameters were reset (figure 2 and table 3) from the onset of germination instead of from seed incubation, the germination components are rather similar, whether germination occurred naturally (non-dormant seeds) or as a result of a Se treatment (dormant seeds). Germination in the two groups was only displaced differently along the abscissa axis, the germination of non-dormant seeds occurring earlier, i. e., towards the ordinate. This similarity suggests that the mechanisms underlying the germination both non-dormant and Se-treated seeds are likely the same. As for the delay for the onset of germination in Se-treated seeds, it can be attributed to the different uptake rates, transport, metabolism, arrival to the target cells (Läuchli, 1993) and Se 'activation' (see below) to leading to dormancy breakage by the several compounds.

Non-dormant Townsville stylo seeds produce 8-10 fold as much ethylene than dormant ones; the same phenomenon was observed in low pH-stimulated seeds (Pelacani *et al*., 2005) and is also shown in table 4. Ethylene-producing compounds such as ACC and CEPA break seed dormancy (Vieira and Barros 1994). The ethylene requirement for seed dormancy breakage was demonstrated recently by Ribeiro and Barros (2006). Thus, like low pH, it is possible that Se compounds trigger dormancy breakage through eliciting ethylene biosynthesis. Pinheiro *et al.* (2008) observed that several Se compounds eliciting dormancy breakage also stimulated ethylene production by the seeds. Their studies were continued, and as shown in table 4, not only dormant bu also Se-treated seeds produced much more ethylene than untreated dormant seeds. Seed germination showed well correlated with ethylene production, suggesting that dormancy breakage by Se-compounds was consequence of their inducing ethylene production.

All evidence points towards a causal relationship between ethylene accumulation and seed dormancy breakage, but the two phenomena could simply be running in parallel. This matter was investigated by attempt to inhibiting ethylene biosynthesis and action. Inhibitors of both biosynthesis (AVG, $Co^{2+}$) and action ($Ag^{+}$) of ethylene largely depressed germination of Se-stimulated seeds (table 5). The involvement of ethylene in the process of dormancy breakage was further confirmed by the fact that germination was restored almost to the full when inhibitors were simultaneously supplied to seeds with ethylene-producing substances (AVG plus ACC, $Co^{2+}$ plus CEPA) or upon transferring $Ag^{+}$-treated seeds to a low pH HCl solution (to precipitate $Ag^{+}$). Altogether these data were expanded and confirmed the results of Pinheiro *et al.* (2008) and pointed to ethylene as a key molecule in the chain of events triggered by Se compounds leading to dormancy breakage.

Although Se compounds break seed dormancy through inducing ethylene production, the relationship between the amounts of ethylene produced (table 4) and $\mathbf{I_{ef}}$ (table 2) is

not so straightforward. The compounds which induced the greatest ethylene production by seeds (SeM and SeU) did not lead to the largest $\mathbf{I_{ef}}$(s). This could be due to additional post-ethylene production factors (e.g. cell division and expansion) also affecting germination. All factors were then summed up and integrated by $\mathbf{I_{ef}}$, showing it to be an integrative index.

Summarizing Se compounds seem to break dormancy of Townsville stylo seeds by triggering ethylene biosynthesis. Concentrations of Se compounds slightly higher than the optimal ones shown in table 1 may cause decreased germination and seedlings produced showed reduced growth (Barros and Freitas, 2001). Decreased germination as caused by Se toxicity was also seen when non-dormant seeds were treated with $Na_2SeO_3$, $H_2SeO_3$, $SeCl_4$ and $SeO_2$ at concentrations equal or higher than 0.1 mM or treated with $Na_2SeO_4$, $H_2SeO_4$, SeU and SeM at concentrations equal or higher than 1.0 mM (not shown). These concentration ranges stimulated dormancy breakage (table 1 and figure 2). We thus believe that a stress condition created by Se toxicity could trigger ethylene biosynthesis as several other stresses do (Pech *et al*., 2004). In non-accumulator plants toxicity brought about by Se is likely a result of its unspecific incorporation into proteins and other compounds in replacement to sulphur (Terry *et al*., 2000, Zhang *et al*., 2007). If this hypothesis holds true the Se of the several compounds had to be somehow 'activated' to create the stressing condition that would trigger ethylene biosynthesis. Thus as already pointed out uptake of the compounds by seed tissues, their transport, a possible metabolism over their pathways, arrival to target cells and the 'activation' of Se might have contributed to produce different moments for the onset of germination promoted by each compound shown in table 3.

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