

### Background

Uranium contamination in the environment has resulted from releases linked with nuclear fuel cycle activities and from industries extracting and processing materials containing naturally occurring radionuclides (e.g. phosphate industry). Uranium toxicity effects are predominantly studied on man and animal species, but little information is available for plants. If phytomanagement of uranium contaminated soils is considered, biological effects on the vegetation have to be investigated. Information on the contamination impact can also be used for risk assessment and derivation of clean-up standards.

Plants can experience oxidative stress when they are exposed to environmental stress situations (e.g. exposure to heavy metals). Reactive oxygen species (ROS) are produced in both stressed and unstressed cells potentially leading to cellular damage. Consequently, plants have developed an antioxidative defence system comprising ROS-scavenging enzymes (e.g. SOD (superoxide dismutase), CAT (catalase) ...) and metabolites (e.g. ascorbate, glutathione ...). Previous results showed that uranium exposure can cause an imbalance between the oxidative and antioxidative capacities of the plant cells.

### Objectives

The present study aimed to analyse biological effects induced in *Arabidopsis thaliana* after bioaccumulation of uranium and to define possible dose-effect relationships. Subtle effects on the antioxidative defence system (enzymes, metabolites ...) viewed as early responses for individual disturbances (growth, nutrient profile ...) were analysed.

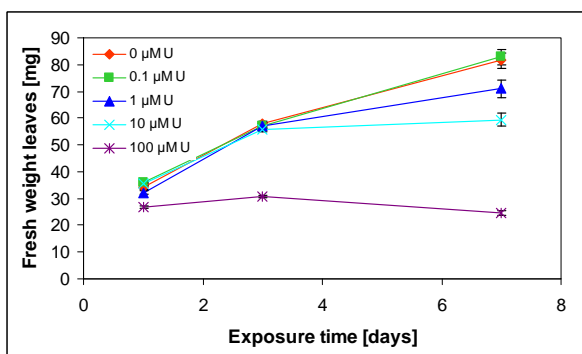
### Principal results

Three-week-old *Arabidopsis thaliana* seedlings were exposed to 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U (uranium) for 1, 3 and 7 days. After harvest, biometric parameters (fresh weight and root length), uranium content, nutrient profile, membrane damage, DNA integrity, enzyme capacities, metabolite concentrations and gene expressions were analysed for leaves and roots.

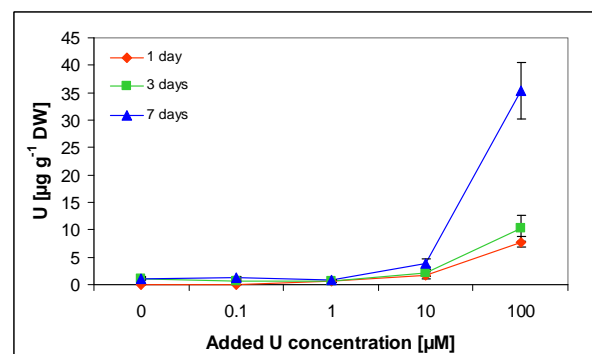
After 7 days' exposure, fresh weight of leaves and roots was significantly decreased for seedlings treated with 100  $\mu\text{M}$  U. After 3 days' exposure to 100  $\mu\text{M}$  U, leaves showed chlorosis and roots were stunted and turned yellow. The percentage dry weight increased for leaves and roots treated for 7 days with 100  $\mu\text{M}$  U indicating that the plants started to wilt. Uranium concentrations were highest in leaves and roots treated with 100  $\mu\text{M}$  U. For roots no time effect was visible suggesting uranium was precipitated on the root surface from the first day of exposure.



*Arabidopsis thaliana* seedlings exposed to different uranium concentrations in hydroponics and harvest of leaves and roots



Fresh weight [mg] of leaves of *Arabidopsis thaliana* seedlings exposed for 1, 3 and 7 days to 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U. Each value represents the mean  $\pm$  S.E. (standard error)

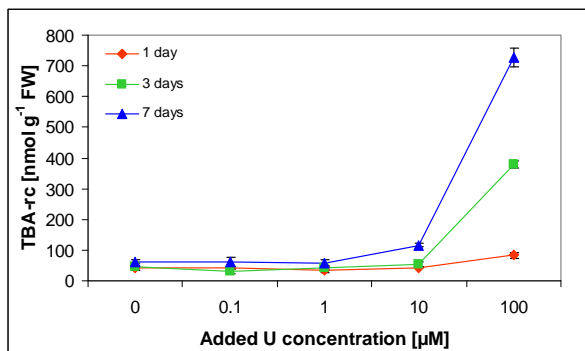


Uranium concentrations [ $\mu\text{g g}^{-1}$  DW (dry weight)] in leaves of *Arabidopsis thaliana* seedlings exposed for 1, 3 and 7 days to 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U

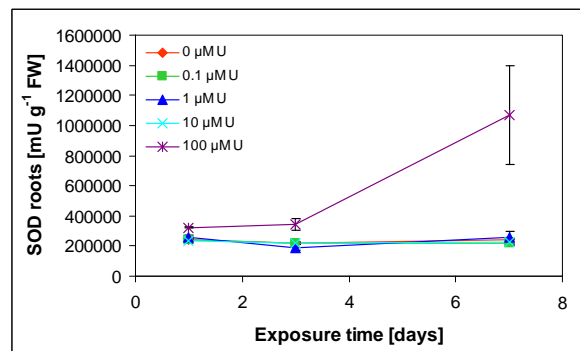
Uranium mostly accumulated in/on the roots and transfer to the leaves was limited suggesting more severe toxicity effects can be expected in roots of *Arabidopsis thaliana* seedlings.

Uranium presence affected the nutrient profile of the plants. A decrease of most element concentrations (e.g. K, Na, S ...) was observed in leaves and roots after exposure to 100  $\mu\text{M}$  U.

An increase in lipid peroxidation products was observed in leaves of *Arabidopsis thaliana* after treatment with 100  $\mu\text{M}$  U affecting membrane integrity and functionality. This was supported by the observed potassium leakage, an indicator of membrane instability.



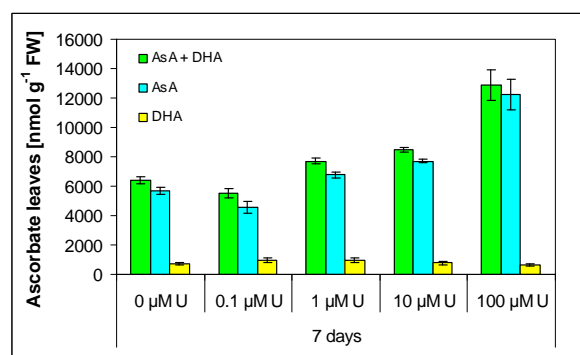
The level of lipid peroxidation based on the amount of TBA-rc (thiobarbituric acid-reactive compounds) [ $\text{nmol g}^{-1} \text{FW}$  (fresh weight)] in leaves of *Arabidopsis thaliana* seedlings treated with 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U for 1, 3 and 7 days



Enzyme capacity [ $\text{mU g}^{-1} \text{FW}$ ] of SOD (superoxide dismutase) in roots of *Arabidopsis thaliana* seedlings exposed to 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U for 1, 3 and 7 days. An enhanced capacity was observed after 7 days' exposure to 100  $\mu\text{M}$  U

Within a cell, SOD's constitute the first line of defence against ROS, they transform  $\text{O}_2^{\bullet-}$  to  $\text{H}_2\text{O}_2$ . Increased SOD capacities were observed in roots treated for 7 days with 100  $\mu\text{M}$  U suggesting an enhanced protection against ROS. After conversion of  $\text{O}_2^{\bullet-}$  into  $\text{H}_2\text{O}_2$ , several enzymes regulate the transformation of  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  (e.g. CAT, GPOD (guaiacol peroxidase), SPOD (syringaldazine peroxidase) ...). Increased enzyme capacities of GPOD and SPOD were visible for roots treated with 100  $\mu\text{M}$  U. The ascorbate-glutathione cycle, comprising several metabolites and enzymes, is also an important pathway in detoxifying  $\text{H}_2\text{O}_2$ . In this study, two enzymes (APX (ascorbate peroxidase) and GR (glutathione reductase)) and two metabolites (ascorbate and glutathione) from the ascorbate-glutathione pathway were analysed. An increase in total ascorbate (AsA (ascorbic acid) + DHA (dehydroascorbate)) was observed in leaves treated for 7 days with 100  $\mu\text{M}$  U. This was due to an increase in AsA (reduced form) but a steady-state situation in the amount of DHA (oxidized form). These results suggest an activation of antioxidative defence via the ascorbate-glutathione pathway in leaves of *Arabidopsis thaliana* seedlings.

Exposure of *Arabidopsis thaliana* seedlings to 100  $\mu\text{M}$  U caused a decreased growth, an unbalanced nutrient profile and membrane damage. It also induced oxidative stress and affected the antioxidative defence mechanism.



Ascorbate content [ $\text{nmol g}^{-1} \text{FW}$ ] in leaves of *Arabidopsis thaliana* seedlings exposed for 7 days to 0, 0.1, 1, 10 and 100  $\mu\text{M}$  U

### Future work

As radionuclides often occur in combination with other contaminants/stressors (e.g. heavy metals, radiation ...), investigation of biological effects induced in *Arabidopsis thaliana* should also focus on this multi-pollution context. Using a multi-biomarkers approach future work aims to investigate the additive/synergistic/antagonistic character of exposure to multiple stressors (uranium and gamma radiation).

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### Main reference

Vanhoudt N., Vandenhove H., Smeets K., Remans T., Van Hees M., Wannijn J., Vangronsveld J., Cuypers A., "Effects of uranium and phosphate concentrations on oxidative stress related responses induced in *Arabidopsis thaliana*". Submitted to Plant Physiology and Biochemistry in December 2007.