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Uranium Effects on the Growth of Soybean (*Glycine max* (L.) Merr.)

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Some naturally occurring radionuclides accumulate in appreciable amounts in plants, adversely affecting their growth (Vavilov et al. 1977; Titaeva et al. 1978; Yastrebov 1978). One such radionuclide is uranium, which is released to the environment from the mining and processing of uranium ores and the increased use of phosphate fertilizers (Menzel 1968; Sackett et al. 1972; McNabb et al. 1979). Fertilizers have become a mainstay in the world's agricultural community. All fertilizers contain various members of the uranium-thorium decay series originating from the phosphate component of the fertilizers (Guimond 1978). Many phosphate rock deposits have been examined for uranium content and concentrations ranging from 30 to 200 µg/g have been reported (Davidson and Atkin 1952; Mazor 1963). High concentrations of uranium have been found in water (Spalding and Sackett 1972) and soil (Johnson et al. 1980), and this element may be taken up by the root systems of plants (Morishima et al. 1977). Because phosphate fertilizers are commonly used in agriculture, there is a need to evaluate uranium uptake by crop species. Hence, the present study was conducted to measure uranium taken up by soybean during germination and early growth and to determine its effects on the germination and chlorophyll content of the leaves.

MATERIALS AND METHODS

Uranium oxide (natural uranium) was obtained from Radiation Protection Bureau, Health and Welfare Canada, Ottawa. Glass-distilled acetone was purchased from Caledon Laboratory Ltd. Georgetown, Ont. Canada. Nitric acid (Reagent A.C.S.), potassium persulfate and sodium hydroxide (A.C.S.) were from the Fisher Scientific Company, New Jersey, U.S.A.

Soybean seeds (*Glycine max* (L.) Merr. var. maple presto) were stored at 5°C until used. Before germination, the seeds were sterilized in 2% hypochlorite solution and washed with deionized distilled water for 30 minutes. The water wash was tested with 1% silver nitrate solution to ensure that the added chloride anion was no longer present.

The seeds were germinated in heat-sterilized Pyrex petri-dishes. Ten comparably sized seeds were placed in each dish, and 20 mL of Hoagland's nutrient solution #2 added (Hoagland and Arnon 1938). Uranium oxide was added to give final concentrations of 0, 0.42 and 42.0 µg/mL uranium. The seeds (100 per treatment) were germinated in the dark at a temperature of 27±2 $^{\circ}$C and a relative humidity of 60%. Experiments were repeated three times for a total of 300 seeds. The data were statistically analysed using one way analysis of variance (Tukey 1949).

The seeds were examined daily and germination was assessed to be complete when the roots pierced the seed coats (Mayer and Poljakoff-Mayber 1963). Germination values (Czabator 1962) were calculated 6d post treatment. At this time, the length of the seedlings were measured from cotyledon to the root tip. The uranium content of the 6d old seedlings was also assayed.

One gram (dry-weight) of the seedlings was dry ashed in a muffle furnace for 2 h at 650 $^{\circ}$C. The ashed residue was dissolved in 25 mL of distilled water. Two grams of potassium persulfate were added and the solution was evaporated to dryness. The dried residue was dissolved in 40 mL distilled water and the pH adjusted to a slightly basic reading (pH 8) with 10 mol/L sodium hydroxide and then the solution was neutralized with 10% nitric acid. The resultant solution was transferred to a 50 mL volumetric flask and diluted with distilled water to 50 mL. Uranium content was determined by laser fluorimetry (Measures and Lecompte 1980).

In a parallel set of experiment, 4d old soybean seedlings were transferred to 250 mL Erlenmeyer flasks containing 100 mL Hoagland's nutrient solution #2 (Hoagland and Arnon 1938). Uranium oxide was added to give concentrations of 0, 0.42 and 42.0 µg/mL uranium. The seedlings were then grown for a further four weeks in an environmentally controlled growth chamber day:night temperature 20:18 $^{\circ}$C, light:dark 12:12 h, (17.2 klx.), and relative humidity 60%. The solutions were changed on days 6, 11, 15, 18, 22 and 26 and the pH of the medium was maintained in the range of pH 5.0-pH 6.5.

Four-week-old soybean plants were harvested for chlorophyll and uranium analyses. The uranium content of the shoots and roots were analysed separately.

0.5 g of ontogenetically similar leaves (L1, L2 and L3) was homogenized in 8 mL of 98% acetone, filtered through Whatman #1 filter paper, and diluted to 80% v/v acetone:distilled water. Optical density readings were made at 663 and 645 nm using a Unicam SP 1800 ultraviolet spectrophotometer and total chl., chl_a and chl_b calculated (Arnon 1949).

RESULTS AND DISCUSSION

The total final germination and the germination values of soybean were not affected by either concentration of uranium (0.42 and 42.0 µg/mL U). Subsequent growth was significantly depressed in the set exposed to 42.0 µg/mL uranium, and this was evidenced by a 33% decrease in seedling length (Table 1).

Table 1. Length (cm) of soybean seedlings after six days of germination.

Treatments	Means±S.E.	% of control
Control	10.0±0.46 a*	
0.42 µg/mL U	9.3±0.45 a	93%
42.00 µg/mL U	6.8±0.38 b	67%

*Means (n = 300) followed by the same letter are not significantly different at 0.05 confidence level, (TUKEY 1949).

The uranium uptake in 6dold soybean seedlings increased with the increasing concentration in the medium (see Table 2).

Table 2. Accumulation of uranium in the seedlings of six day-old soybean (n = 6).

Treatments	µg U/g dry wt.±S.D.
Control	0.41±0.14
0.42 µg/mL U	1.96±0.23
42.00 µg/mL U	110.50±6.50

Uranium uptake following 4 weeks of growth also indicated that uptake was increased with the increasing concentration of uranium in the medium. The accumulated uranium was preferentially located in the root system. The ratio of uranium in the roots:shoots was as 10:1 at the higher concentration (Table 3). Similar results were recorded by Prister and Prister (1970) and Cannon (1960). These results are consistent with the fact that uranium forms dissociable complexes with certain active groups on the cell surface (Rothstein et al. 1948; Rothstein and Larrabee 1948). The active complexing groups may be carboxyl, hydroxyl or phosphate (Rothstein and Meier 1951). Binding with such groups on the root cell surfaces, coupled with an inability to penetrate the endodermal barrier, could readily lead to the presently observed accumulation of uranium in the roots apoplast.

The toxic symptoms induced by uranium in 4wk old soybean plants included chlorosis, early leaf abscision, a general reduction in root growth, and at the higher treatment level, a wide spread necrosis of tissue (Fig. 1).

Table 3. Accumulation of uranium in the shoot and root system of four-week-old soybean plants (μg U/g dry wt.±S.D.) (n = 6).

Treatments	Shoot	Root
Control	0.62±0.03	4.09±0.21
0.42 μg/mL U	1.37±0.07	57.01±2.90
42.00 μg/mL U	91.51±4.50	938.10±22.6

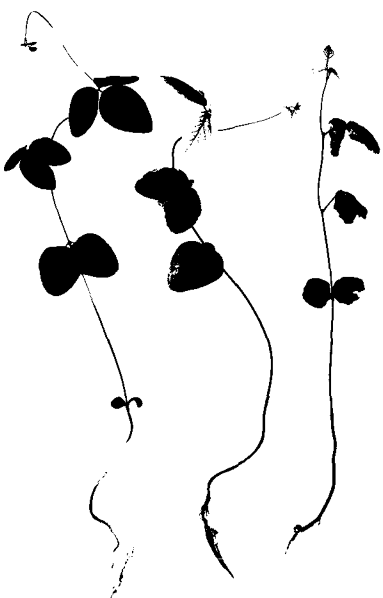

Fig. 1 Four-week-old soybean plants: left to right: Control, treated with 0.42, and 42.0 μg/ml uranium.

The effect of uranium (0.42 and 42.0 μg/mL U) on the total chl, chl_a and chl_b is shown in Table 4 where chlorophyll is expressed as percentage of control. In plants treated with 0.42 μg/mL U, total chl, chl_a and chl_b were not decreased significantly in any of the leaves. However, a significant 30 to 40% decrease in total chl, chl_a and chl_b was observed in leaf 2 and leaf 3 exposed to 42.0 μg/mL U.

Generally, more uranium was found in the roots than in the shoot systems although the content ratio was variable. In the present investigation, the severe reduction in root length can be conceived as leading to a

Table 4. Mean total chlorophyll, chl_a and chl_b from the leaves of four-week-old soybean plants (% of control).

Treatments	Total chl.		Chl_a		Chl_b	
Leaf 1						
0.42 µg/mL U	81±5	a*	82±5	a*	77±6	a*
42.00 µg/mL U	75±13	a	76±13	a	72±15	a
Leaf 2						
0.42 µg/mL U	76±9	a	77±9	a	74±11	a
42.00 µg/mL U	58±17	b	59±16	b	55±20	b
Leaf 3						
0.42 µg/mL U	93±21	a	93±18	a	96±28	a
42.00 µg/mL U	64±12	b	68±12	b	58±10	b

*Means (n = 6) followed by the same letter are not significantly different at 0.05 level, (Tukey 1949).

reduction in the absorptive capacity; this, coupled with the possible disfunction of xylem and phloem tissues due to uranium precipitation (Cannon 1960) and complexing, might have been the major causes of the early onset of necrosis accompanied by reduced leaf chlorophyll.

The present study clearly demonstrates that uranium is absorbed by plants. This may be the cause of the severe reduction of root growth and leaf chlorophyll content observed. In the present study, the uranium content in soybean increased with increasing uranium concentration in the medium. The accumulated uranium was preferentially located in the roots.

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