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To: R. Sauer, NASA-JSC (SD-4)
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RE: PLANT SEEDLING RESPONSE TO IODINATED WATER

Introduction

Iodine has been used to disinfect potable water on US spacecraft since Apollo (1). A Microbial Check Valve (MCV) is currently used to impart 2 parts per million (ppm = mg/l) of iodine to potable water (2,3). It is likely the US Space Station and other long-duration missions will utilize and eventually recycle iodine-disinfected water.

MCV water causes measurable physiological changes in human consumers, and may cause chronic toxicity effects with long term consumption (4). Review of current agricultural, biological and federal research literature yielded virtually no experimental data on the effect of iodinated water on plants.

Materials and Methods

All glassware was cleaned, triply-rinsed with Class III glass-distilled water (GDW), and where appropriate terminally washed with iodinated water (I2W) prior to use. I2W was prepared by passing GDW through a newly-charged MCV (Umpqua Research Company, Myrtle Creek OR). GDW and I2W were stored in tetrafluoroethylene-sealed, glass bottles.

Seed packets of ten higher plant species currently used for microgravity experimentation and space food production (Table 1) were obtained (Northrup King Seed Company, Minneapolis MN). All seeds for an individual species were taken from a single seed packet. Abnormal-appearing seeds were discarded. Equal numbers of the remainder were transferred into paired, 250 ml, glass, Erlenmeyer flasks. Each flask entrance was then covered with 0.5 mm internal mesh, tetrafluoroethylene screen (Spectrum Medical, Carson CA) and the screens secured tightly with a rubber band. Fourty ml aliquots of GDW and I2W were transferred into respective flasks using dedicated, all-glass syringes. Seeds were soaked for 4 hours, drained, and rewetted with respective solutions for 10 minutes approximately every 12 hours. Between rewettings, flasks were randomly reassembled into a tight square and stored in an area protected from direct ventilation currents, within an approximately 12 foot by 18 foot, constantly incadescently illuminated, windowless room. Seeds were observed prior to each rewetting for first appearance of primary leaves. On appearance, all seeds of that particular species were examined for the presence of plant tissue (germination). Germination rates were calculated for experimental (I2W) and control groups. Data were analyzed using the standard Chi-square statistic at 90% confidence.

On about the sixth day, illumination, dry temperature and intra-flask humidity were measured for corner and middle flasks. Illumination and temperature were measured at the top of the flasks with a Weston #267779 Foot Candle Meter (Weston Instruments, Newark NJ) and ASTM #63C mercury/glass thermometer (Fisher Scientific, Pittsburg PA). Intra-flask humidity adequacy was determined by observing for the presence of visible water around seeds immediately before rewetting.

Approximately twelve days after the experiment, a representative sample of I2W was drawn from the I2W storage bottle using the dedicated syringe, and analyzed for IDP concentrations using a Shimadzu UV-265 scanning visible UV spectrophotometer (Shimadzu, Kyoto, Japan).

Results

First primary leaves were observed in *Raphanus sativus* (radish) and the *Brassica* sp (broccoli, turnip, cabbage and cauliflower) at 99.4 hours (4.1 days), in *Glycine max* (soy bean) and *Phaseolus vulgaris* (pole bean) at 175 hours (7.3 days), and the remainder (corn, carrot and lettuce) at 194 hours (8.1 days). Germination rates for I2W and control groups for all seeds together were 79.41% (648/816) and 85.66% (699/816) respectively (Chi-square = 11.06, $p < .005$, df = 1). Germination rates and Chi-square values for I2W verses GDW groups by species are summarized in Table 1.

TABLE 1 - GERMINATION RATES FOR TEN HIGHER PLANT SPECIES WETTED WITH MCV (SHUTTLE IODINATOR) -IODINATED WATER (I2W) AND GLASS DISTILLED WATER (GDW) (NASA, 1988).

	Germination rate (%)		
Genus/Species/Cultivar	I2W	GDW	Chi-sq
Glycine max	78.9 (41/52)	100.0 (52/52)	10.6008*
Brassica oleracea italica	15.0 (15/100)	32.0 (32/100)	8.0378*
Phaseolus vulgaris	89.6 (86/96)	99.0 (95/96)	7.8112*
Zea mays	95.2 (40/42)	100.0 (42/42)	2.0488
Brassica oleracea botrytis	84.6 (22/26)	96.2 (25/26)	1.9915
Daucus carota	82.0 (82/100)	87.0 (87/100)	0.9544
Brassica oleracea capitata	81.0 (81/100)	85.0 (85/100)	0.5670
Brassica campestris rapisera	95.0 (95/100)	97.0 (97/100)	0.5208
Lactuca sativa	87.0 (87/100)	88.0 (88/100)	0.0457
Raphanus sativus	99.0 (99/100)	96.0 (96/100)	1.8462

* Significant at 90% confidence ($p < .10$, Chi-sq > 2.71, df = 1)

Illumination and temperature measurements at corner and middle flasks were all 20.0 foot-candles at 22.1 degrees Centigrade. Intra-flask humidity was judged adequate for all flasks.

I2W was determined to contain 1.09 ppm iodine, 0.49 ppm iodide and 0.009 ppm tri-iodide.

Discussion

Germination rates were significantly diminished in I2W compared to GDW exposed seeds for all species taken together, and for _Glycine_ _max_ (soy bean), _Brassica_ _oleracea_ _italica_ (broccoli), and _Phaseolus_ _vulgaris_ (pole bean) in order of descending significance. All other species except _Raphanus_ _sativus_ (raddish) followed this trend. Beans were most affected.

While I2W germination rates were significantly diminished for broccoli, I2W and control rates were both surprisingly low. Other _Brassica_ sp did not show similar magnitude reductions in either experimental or control rates.

Anecdotally, I2W seeds appeared to germinate slightly sooner than GDW seeds. I2W seeds also appeared to be developmentally more mature, and in some cases to be greater in overall length than controls.

Inter-flask variations in ventilation, illumination, temperature and humidity were not detected within the limits of the methods employed. Determinations were not repetitive, as differences between experimental and control flasks rather than group changes over time were of most importance. Extreme group environmental changes could theoretically induce stress reactions in seedlings, which might augment or diminish the experimental seedlings' response to iodination products.

The presence of iodine, iodide and tri-iodide in I2W complicates any attempt to ascribe observed differences specifically to iodine.

Glass flasks and tetrafluoroethylene screens were used to minimize material iodine uptake. Actual uptake by materials and plants was not measured. However, _ex_ _post_ _facto_ spectrophotometric data suggest that time, materials and delivery technique did not result in substantial iodine species loss.

The above data are not inconsistent with pre-1950 literature (5). Analysis of this literature prompted the Chilean Iodine Eductional Bureau to state that "...the more iodine there is available the more a plant will absorb, until toxic levels are ultimately reached (5)." In addition, water culture experimental data were interpreted to show that

> ...no [plant] species withstands a concentration greater than 1 part of iodide in 1 million parts of solution. Indeed, at this strength the growth of peas and mustard is retarded, and any higher concentration is definitely harmful. Only when the concentration is reduced to 1 part of iodide in 5 or 10 million parts of solution has any favourable stimulatory effect been observed (5).

A consistent trend towards decreased germination rates in MCV-water wetted seeds was noted for most seeds. It is probable, therefore, that small additional increases in iodine disinfection

PLATE 1 - Glycine max (soy bean) after appearance of primary leaves.

1) In glass distilled water (GDW)
2) In MCV (iodinated GDW)

PLATE 2 - G. max sprouted in GDW

- largest / best developed
- representative
- smallest / least developed

PLATE 3 - G. max sprouted in MCV

- largest / best developed
- representative
- smallest / least developed

NOTE SIMPLIFIED ROOTLETS

PLATE 4- Phaseolus vulgaris (pole bean) after appearance of primary leaves

11) In GDW
12) In MCV

PLATE 5- P. vulgaris sprouted in GDW

(Right to Left):

- largest / best developed
- representative
- smallest / least developed

PLATE 6- P. vulgaris sprouted in MCV

(Right to left):

- largest / best developed
- representative
- smallest / least developed

NOTE SIMPLIFIED ROOTLETS..

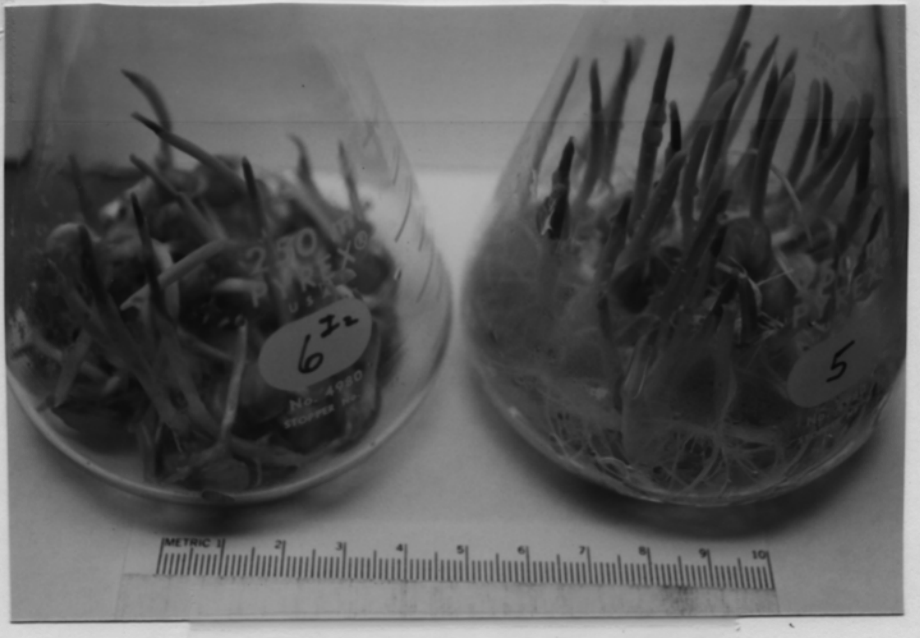

PLATE 7 – *Zea mays* (corn) after appearance of primary leaves

5) In GDW

6) In MCV

PLATE 8 – *Z. mays* sprouted in GDW

- largest / best developed
- representative
- smallest / least developed

PLATE 9 – *Z. mays* sprouted in MCV

- largest / best developed
- representative
- smallest / least developed

products as might occur in water recycling, closed environmental life suport systems, and/or space gardening, might heighten observed effects.

Since the iodine disinfection species causing the above effects is not known, and since the plants were only observed through primary leaf emergence, the effect of I2W on mature plants and consumers of such plants is unknown.

Conclusion

Our data do not support the generally held belief that plants are insensitive to low concentrations of iodine in water. Some plants, beans in particular and possibly Brassica sp, appear sensitive to MCV water. In light of these findings, further investigation seems indicated.

Acknowledgement

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