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EFFECT OF IODINE DISINFECTION PRODUCTS ON HIGHER PLANTS

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ABSTRACT

Iodine is used to disinfect potable water on United States spacecraft. Iodinated potable water will likely be used to grow plants in space. Little is known about the effects of iodine disinfection products on plants. Seeds of select higher plants were germinated in water iodinated using the Shuttle Microbial Check Valve, and water to which measured amounts of iodide was added. Percent germination was decreased in seeds of most species germinated in iodinated water. Beans were most affected. Germination rates, determined from germination half-times, were decreased for beans germinated in iodinated water, and water to which iodide was added. Development was retarded and rootlets were conspicuously absent in bean and several other plant species germinated in iodinated water. Iodide alone did not elicit these responses. Clearly iodine disinfection products can affect higher plants. These effects must be carefully considered for plant experimentation and cultivation in space, and in design and testing of closed environmental life support systems.

INTRODUCTION

Iodine has been used to disinfect potable water on US spacecraft since Apollo /1/. A Microbial Check Valve (MCV; Umpqua Research Company, Myrtle Creek, OR, USA) is used to impart 2 parts per million (ppm = mg/l) of iodine to potable water /2,3/. Crews of future missions (for example, US space station) as presently conceived will reuse and recycle MCV-iodinated water. Future crews and, as such, consumers, will undoubtedly include plants.

At these concentrations, iodine can cause measurable physiological changes in human consumers and may be toxic over long periods /4/. The effects of iodinated water on plants are less well known. Plants are not known to have an iodide-based hormone system as do humans and animals. Iodine and iodide are considered by some plant physiologists to be nontoxic and possibly even non-essential to plants /5/. However, after critical review of the literature, at least one group has concluded that the more iodine there is available the more a plant will absorb, until toxic levels are ultimately reached /6/. Aquaculture data further suggest 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 /6/."

We examined germination numbers and rates of seeds sprouted in glass distilled (GDW), MCV-iodinated (I2W), sodium iodide (NaIW) and potassium iodide (KIW) water.

METHODOLOGY

All glassware was multiply rinsed with Class III GDW, and where appropriate multiply rerinsed with I2W prior to use. I2W was prepared by passing GDW through a newly-charged MCV, and was scanned from 210 to 750 nm with a scanning visible UV spectrophotometer (Shimadzu, Kyoto, Japan) for specific iodine disinfection products (IDP). I2W was stored in dark, tetrafluoroethylene (TFE) sealed, glass bottles.

Percent Germination (% GERM) Experiment

Seeds of ten higher plant species important for plant physiological experimentation, food or life support in space were obtained (Table 1; Northrup King Seed Company, Minneapolis, MN, USA). All seeds for a particular species were from the same 1988 seed lot. Abnormal-appearing seeds were discarded. Remaining seeds were equally distributed between two 250 ml Erlenmeyer flasks. Flask entrances were then covered with 0.5 mm internal mesh, TFE screens (Spectrum Medical Company, Carson, CA, USA) and secured with rubber bands. Seeds were soaked for 4 hours in either I2W or GDW, drained, and rewetted with respective solutions for 10 minutes approximately every 12 hours. Between rewettings, flasks were randomly reassembled into a tight square and placed in a protected area in a large, windowless, fluorescently-illuminated room. Upon first appearance of any primary leaves, all seeds of that species were harvested and examined for visible plant tissue (germination). % GERM's were calculated and analyzed using a standard Chi-square statistic at 99% confidence.

Germination Half-time (GERM 1/2) Experiment

Using the above techniques, multiple serial dilutions of NaIW and KIW were made up, scanned and solutions with an iodide concentration similar to I2W identified.

Seeds of *Glycine max*, *Zea mays* and *Triticum aestivum* were inbibed in I2W, NaIW, KIW and GDW, transferred onto TFE screens inside inverted petri plate covers, covered with petri plate bottoms, and incubated in a plant growth chamber for 5 days. Seeds were automatically photographed every 2 hours on 70 mm Kodak Tri-X film (Eastman Kodak Company, Rochester, NY, USA). Plates were maintained at 24 degrees Centigrade inside the chamber using a temperature-controlled, circulating water bath. The chamber was constantly illuminated with fluorescent light at 20 micromols/square meter-second. Photographs were analyzed using low power stereo dissecting microscopy using a slide viewing box for illumination. Cumulative % GERMS were calculated, plotted, and GERM 1/2's were determined graphically and finally verified by linear regression extrapolation.

RESULTS

I2W was determined spectrophotometrically to contain at least iodine, iodide and tri-iodide IDP species.

% GERMS were significantly lower in I2W compared to GDW exposed seeds for all species taken together, and for *Glycine max* (soybean), *Brassica oleracea* cv italica (broccoli), and *Phaseolus vulgaris* (pole bean) in order of decreasing significance (Table 1). Other seeds, except *Raphanus sativus* (raddish), showed the same trend, but were not statistically significant. Clearly, beans were most affected.

On further examination, several other differences were noted. Soybeans, pole beans and broccoli exposed to I2W germinated faster than GDW controls, while *Zea mays* (corn), and *Brassica oleracea* cv capitata (cabbage) germinated slower and were shorter than GDW controls. Soybeans, pole beans and corn exposed to I2W appeared, however, to be less developmentally mature, and to have strikingly fewer rootlets than GDW controls.

GERM 1/2's from the second experiment are summarized in Table 2. The GERM 1/2 for I2W-exposed soybeans was about half that for GDW-exposed controls. GERM 1/2's for NaIW and KIW exposed soybeans were intermediate between I2W and GDW exposed seeds. Similar differences were not observed for corn or *Triticum aestivum* cv yecora rojo (wheat). In this experiment, corn cultures were serendipitously retained to primary leaf formation. As noted in the first experiment, I2W-exposed corn seedlings appeared to be less developmentally mature and to have fewer rootlets than NaIW, KIW or GDW exposed seeds.

TABLE 1 Percent Germination (% GERM) of Select Seeds Sprouted in Iodinated (I2W)* and Uniodinated (GDW) Water

Species/Cultivar	% Germ I2W	GDW	Statistic Chi-sq
Glycine max	78 (41/52)	100 (52/52)	10**
Brassica oleracea cv italica	15 (15/100)	32 (32/100)	8.0**
Phaseolus vulgaris	89 (86/96)	99 (95/96)	7.8**
Zea mays	95 (40/42)	100 (42/42)	2.1
B. oleracea cv botrytis	84 (22/26)	96 (25/26)	2.0
Daucus carota	82 (82/100)	87 (87/100)	1.0
B. oleracea cv capitata	81 (81/100)	85 (85/100)	0.6
B. campestris cv rapisera	95 (95/100)	97 (97/100)	0.5
Lactuca sativa	87 (87/100)	88 (88/100)	0.1
Raphanus sativus	99 (99/100)	96 (96/100)	1.9
All species	79 (648/816)	85 (699/816)	11**

* GDW passed through Shuttle Microbial Check Valve
** Significant at 99% confidence (p < .01, Chi-sq > 6.63, df = 1)

TABLE 2 Germination Half-times (GERM 1/2) for select Seeds Sprouted in Iodinated (I2W)*, Iodated (NaIW and KIW)** and Uniodinated (GDW) Water

Species/Cultivar	I2W	GERM 1/2 (hours) NaIW	KIW	GDW	Statistic Z (one-tail)
G. max***	58 @ 8	71 @ 7	72 @ 11	78 @ 8	-3.5 (p<0.01)
Z. mays	26	27	25	26	n/a
T. aestivum cv yecora rojo	16	18	16	18	n/a

* GDW passed through Shuttle Microbial Check Valve
** 5 ppm iodide as sodium iodide or potassium iodide in GDW
*** N=4, @ = plus or minus one standard deviation

DISCUSSION

Higher plants are affected by IDP's. Of plants examined, soybeans, pole beans and corn were most affected. Not all affected species appeared to be affected the same way.

IDP's specifically affected seed germination, growth and development. Some but not all of these effects are attributable to iodide. However, the presence of iodine, iodide, tri-iodide and probably other IDP's in MCV-iodinated water complicates any attempt to ascribe observed differences to specific IDP's other than iodide. IDP's are unique water "contaminants" in that they are purposefully added to disinfect potable water. Further elaboration regarding their effects and toxicity is clearly warranted.

Both bean germination experiments produced an acrid, formaldehyde-like odor. It is possible that substance(s) associated with this odor could have been responsible for differences in % GERM and GERM 1/2 noted, if, for example, they were metabolic inhibitors and were produced in quantities proportional to germination rates.

Soybean germination is influenced by a number of environmental cofactors. Several merit special discussion.

Relatively pure, corrosive GDW could have caused seed or seed coat damage in controls. In the former case, differences in GERM 1/2's would be less significant. In the later case, if cell hydration were augmented, differences could be more significant.

Any bacteria present which participate in germination may have been effected by iodine disinfection products. Pure glass distilled water, iodine, and possibly some iodine disinfection products might decrease bacterial types or numbers, while iodide, triiodide and possibly some other iodine disinfection products might support or increase bacterial types or numbers. Effects, if any, on GERM % or GERM 1/2 are difficult to predict.

Humidity and aeration, while the same for experimental and control seeds, probably varied during the course of the experiments. Seeds at different developmental levels due to different germination rates could react differently to variations in humidity or oxygen, causing exaggerated results.

In neither experiment was the effect of I2W on mature plants, their progeny, or consumers of such plants examined.

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