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# The Path of Carbon in Photosynthesis - XXX - $\alpha$ -Mannosides

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Additional information is available at the end of the chapter

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## 1. Introduction

For decades, discoveries have been reported in the series, *The Path of Carbon in Photosynthesis*, including a chapter elucidating a competitive mechanism for binding and releasing sugars from lectins [1], and we present current research that further supports this mode of activity. From the inception of the series [2] and onward, the program has been based on interdisciplinary discourse resulting in achievements of the first order [3], legendary advances of the Path including publications describing the initial products of carbon fixation and with diagrammatic summarization [4]. As a result of the search for carbon fixation intermediates by feeding single carbon fragments ( $C_1$ ) from  $^{14}C$ -methanol to *Scenedesmus* and *Chlorella*, methanol was later applied to improve the growth rate of "Showa" [5]. Colonies of "Showa" were proven to accumulate *in vitro* concentrations of 30% to 40% botryococenes, the highest in the field of hydrocarbon sources for renewable automobile and aviation fuels [6,7] and, as an adjunct to  $C_1$ -cultivation, foliar applications of 15 Molar  $C_1$  formulated with fertilizers were developed [8] and independently verified [9,10]. Consistent with field observations, foliar  $C_1$  inhibited glycolate formation [11]; and thereafter, the application of nuclear magnetic resonance to follow *in vivo* metabolism of methanol identified methyl- $\beta$ -D-glucoside (MeG) [12]. As a consequence of our survey of substituted glycosides, it was shown that not only do glycosides improve productivity, but they also are transported in plants from root to shoot and from shoot to root [13-15]. Furthermore, formulations of polyalkylglycoside and mixed polyacylglycopyranose (MPG) were far more potent than MeG [1].

Having established a history of consistent responses to these substrates, we had often taken note of significant differences that were clearly distinguishable to the naked eye; and so we sought methods to photodocument the events by developing systems for cultivation in glass microbeads ( $\mu$ Beads). Thus, we present images of plants treated with indoxyl- $\beta$ -D-glucoside

(IG) as compared to controls. Previously, significant responses of plants to IG had been reported [15,16]; therefore, in our applications of  $\mu$ Beads, the purpose of this section is to exhibit responses of representative plants without further statistical treatment. In addition to serving as solid support media,  $\mu$ Beads refract light to the improvement of photosynthetic efficiency. Not only does the boost to solar intensity from  $\mu$ Beads have the potential to improve productivity, when increased to saturation, it can have the opposite effect of inhibiting growth by photorespiration. Therefore, in consideration of the critical balance that must be achieved, we cultivated plants in  $\mu$ Beads with safeners, selecting appropriately structured substituted sugars.

$\alpha$ -Glycosides have higher binding affinities to lectins over  $\beta$ -glycosides, therefore, we undertook experiments comparing mixed  $\alpha$ - and  $\beta$ -anomers to  $\alpha$ -mannosides. Mannose polyacetates and methyl- $\alpha$ -D-mannoside were applied to plants because they are closely related to compounds for which we had established dosing. Additionally, responses to low concentrations of arylglucosides, such as IG, provided a starting range of dose requirements for an arylmannoside; and consistent with our hypothesis for specific affinities of lectins, we discovered the highest potencies with  $\mu$ M  $\alpha$ -mannosides.

## 2. Materials and methods

Plants were cultured in research facilities according to previously described methods [1]. Consistency of response to treatments was achieved by supplementation with chelated Ca and Mn. Solutions for foliar applications included phytobland surfactants, but formulas for roots did not. Controls were placed in the same location and all plants were given identical irrigation, fertigation, and handling. Plants were matched to control populations, treated within a week of emergence of cotyledon and true leaves. After treatment, individual plants were scheduled for harvest and analysis. For biomass, plants were dried overnight and weighed. The performance of compounds was evaluated by comparing statistical means of individual dry weights of shoots and roots. All plants were regularly given modified Hoagland water-culture nutrients [17]. Foliar spray applications of identical volumes, either 100 or 186 liters/hectare (L/ha), were mechanically applied. Manual sprays were spray-to-drip volumes of approximately 800 L/ha. For all populations, means of different treatment groups were compared using two-tailed Student's t-test with  $p$ -values significant within 95% confidence intervals. Counts of populations are "n" values and standard error is denoted " $\pm$ SE." Specialty chemicals from Sigma (St. Louis, MO, USA), included the following: tetramethyl- $\beta$ -D-glucoside (TMG); tetraacetyl-D-glucopyranose (TAG); pentaacetyl- $\alpha$ -D-mannopyranose (MP);  $p$ -amino-phenyl- $\alpha$ -D-mannoside (APM); methyl- $\alpha$ -D-mannoside (MeM) and methylglucoside (MeG). MPG was synthesized with modification [1,18]; and 2,3,4,6-tetra-O-acetyl-D-mannopyranose mixed  $\alpha$ - &  $\beta$ -anomers was from Toronto Research Chemicals, North York, ON, Canada. As required, MP and APM were dissolved in a lower aliphatic alcohol prior to dilution in aqueous media. Vascular plants included Canola Nexera 500, *Brassica napus* L., a shoot crop; radish 'Cherry Belle' *Raphanus sativus* L., a root crop; rice, *Oryza sativa* L., a cereal crop; corn TMF 114, *Zea mays* L. ssp. *Mays*; ryegrass, *Lolium multiflorum* Lam., cv Gulf; paperwhite, *Narcissus papyra-*

*ceus* Ker Gawl; ornamental coleus, *Solenostemon scutellaroides* (L.) Codd; and these species were maintained as previously described [13,16].

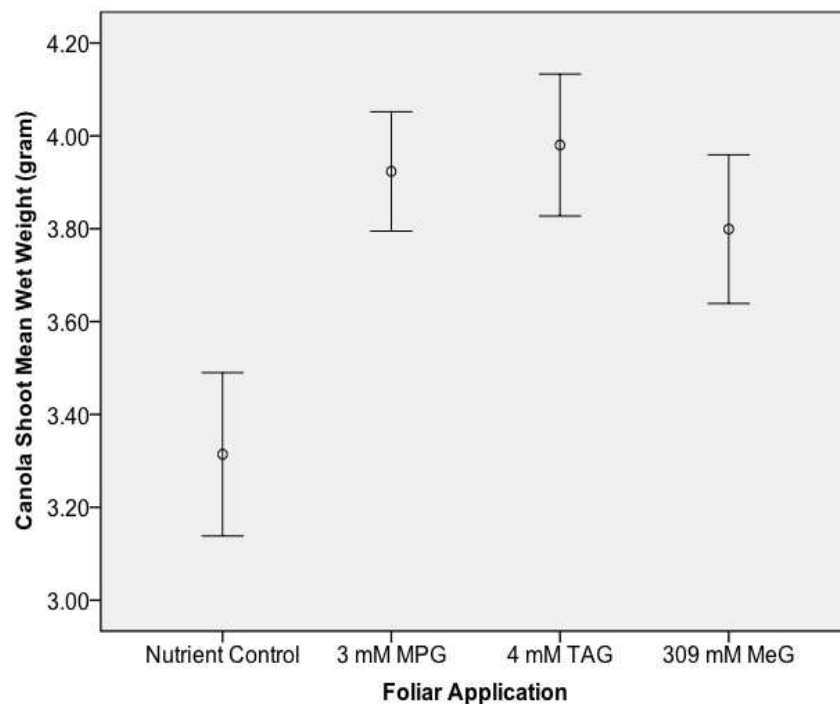
**Rapid Radish Assay** –Radiolabeled methylglucoside is transported into leaves within minutes [14], therefore, we developed a bioassay that could be run within a few days. Furthermore, in the course of surveying different species of plants, we observed germination of radish within 24 h. Therefore, we treated radish with substituted  $\alpha$ -mannosides in water culture nutrients after emergence of hypocotyls as a means of testing our lectin cycle. Radish was sown on 25 - 30 cm Pyrex® dishes with lids or 150 X 15 mm polystyrene Petri dishes filled to depths of seeds with ½X modified Hoagland water-culture nutrients until emergence of hypocotyls. The nutrient solution served as the stock diluent and nutrient control. Overnight, approximately a fifth of the population germinated and those that had shed seed coats were selected for trials. Sprouts were matched for size of cotyledons and hypocotyl and were transferred to Nutrient Control solution or experimental  $\alpha$ -mannosides. In glass dishes, experiments with MP were undertaken completely in water-culture solution. Experiments with APM in water-culture solution were undertaken by sowing sprouted radish seeds on filter paper moistened with treatment or Nutrient Control solutions. Assays were maintained under environmental conditions as follow: Photosynthetically active radiation 100  $\mu\text{Ein m}^{-2} \text{s}^{-1}$ , diel cycle of 16:8 h light:dark, 26:26° C.

**Glass microbeads** - Materials and methods for utilization of clear glass microbeads ( $\mu\text{Beads}$ ) were previously described [16]. Briefly,  $\mu\text{Beads}$  were obtained with the following specifications: Nominal modal diameters 500 - 700  $\mu\text{m}$ ; density 2.5 g/cc; pH 9; and sodalime glass. Reference to the size of a  $\mu\text{Bead}$  refers to its  $\mu\text{m}$  diameter. Reflected light intensity (I) was measured out-of-doors directly over bare sandy loam as compared to 1 cm layer of  $\mu\text{Beads}$  where solar I was in the range of 1700 to 1800  $\mu\text{Ein m}^{-2} \text{sec}^{-1}$ . For drainage, containers were perforated with holes smaller than the  $\mu\text{Beads}$ . Prior to sowing seeds,  $\mu\text{Beads}$  were saturated with pH 6 “nutribead” (modified Hoagland) solution [17] for drip fertigation (<1 L/h) or hourly misting. After >8 h uptake of treatments, fertigation resumed in a manner consistent with pH-control and cultures were regularly given equal volumes of nutribead solution. Controls were placed side-by-side and cultivated likewise. Basal plates of bulbs were immersed into moistened 700  $\mu\text{m}$   $\mu\text{Beads}$  to initiate rooting, after which they were treated. For photography,  $\mu\text{Beads}$  were saturated with water and individual plants were manually lifted out. When roots were dipped in a beaker of water, most of the  $\mu\text{Beads}$  dropped off. Representative plants were selected visually from among experimental populations for macrophotography. To avoid injury from dehydration, plants were photographed within a minute and returned to water.

### 3. Results

The investigations include summaries of previously described experiments with poly-substituted glycopyranoses formulated with nutrients [1]. Manual spray-to-drip foliar treatments were applied to even stands of 5 cm tall radish, as follow: Nutrient Control with 1 g/L surfactants; and 0.3 mM TMG and 1 mM TMG with 1 g/L surfactants. Foliar applications

of 1 mM TMG to radish shoots resulted in a significant ( $n=36$ ;  $\pm SE$  0.07;  $p=0.05$ ) 27% enhancement of mean weights of roots over those of the Nutrient Control. The low concentration of 0.3 mM TMG showed no significant difference ( $n=36$ ;  $\pm SE$  0.05;  $p=0.8$ ) from Control in either growth of radish shoots or roots. The test was calibrated to deliver a volume typical for row crops, 186 L/ha, as follows: Nutrient Control as compared to 3 mM TMG, identically supplemented. The application of foliar 3 mM TMG resulted in a significant ( $n=18$ ;  $p=0.03$ ) increase over the Nutrient Control. TAG is similar to TMG except that it is substituted around the pyranose-ring with four acyls instead of alkyls. Foliar 10 mM TAG and Nutrient Control solutions were applied to shoots of radish and harvested a week later and results showed a significant ( $n=11$ ,  $p=0.004$ ) 27% increase of root mean dry weight as compared to Nutrient Control. The growth response of the roots of radish to foliar TAG, therefore, was similar to that of TMG. Tests were extended to various species of plants and, on Canola, responses to foliar applications of 3 mM MPG, 4 mM TAG and 309 mM MeG were compared. Results are graphically depicted in Figure 1. Three treated populations each showed significant ( $p=0.000$ ) shoot wet weight increases over Nutrient Control, as follow: 3 mM MPG  $n=37$ , 18% increase; 4 mM TAG,  $n=35$ , 20% increase; and 309 mM MeG,  $n=36$ , 14% increase. Foliar 3 - 4 mM polyacetylglucopyranoses showed activity comparable to the higher dose of 309 mM MeG.

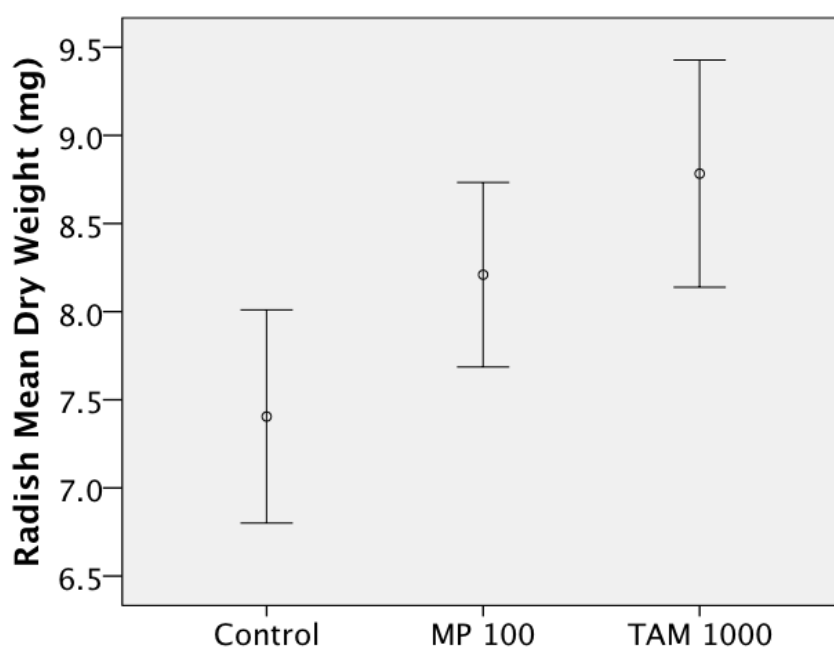


**Figure 1.** Foliar applications with low concentrations of polyacetylglucopyranoses, 3 mM MPG and 4 mM TAG, were comparable to treatments with high methylglucosides, 309 mM MeG, resulting in significant shoot enhancements over Nutrient Control. Error bars indicate  $\pm SE$ .

Treatment of rice with the application of MPG to roots was compared to MeG. Roots exposed to formulations of 500  $\mu M$  MPG and 50 mM MeG showed significant ( $n=27$ ;  $p=0.000$ ) increases in shoot yields of approximately 15% over controls. Roots of corn immersed in 1 mM MPG

were compared to Nutrient Control, individual shoots were harvested after two weeks, and results showed significant ( $n=21$ ; mean dry weight  $p=0.00$ ; mean wet weight  $p=0.000$ ) increases of 12% in vegetative yields of shoots over the population of Nutrient Control.

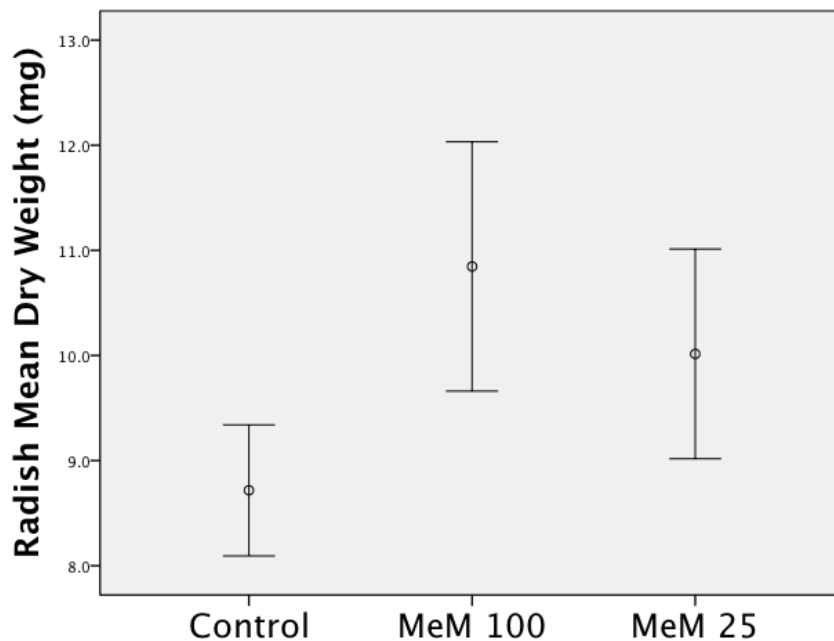
**Rapid Radish Assay** – Methods with radish enabled repetitious runs in the laboratory to determine the range of effective doses and to yield robust data. As mixed  $\alpha$ - and  $\beta$ -anomers, TAM was compared to the  $\alpha$ -anomer, MP. Within one day of exposure to 1 mM TAM or 100  $\mu$ M MP, early greening of the cotyledon leaves was visually discernible from leaves of the Nutrient Control. After 48 h, sprouts treated to 1 mM TAM or to 100  $\mu$ M MP showed advanced growth responses as compared to the nutrient Control. Application of 1 mM TAM in water-culture to radish sprouts resulted in statistically significant enhancement of mean dry weight ( $n=41$ ; 8.8 mg) of whole plants over mean dry weight of the nutrient Control ( $n=41$ ; 7.4 mg;  $p=0.002$ ). At a lower dose, treatment with 100  $\mu$ M TAM resulted in no significant difference of mean dry weight ( $n=41$ ; 8.1 mg;  $p=0.11$ ) from the nutrient Control. Application of MP to radish sprouts resulted in significant enhancement of mean dry weight ( $n=41$ ; 8.2 mg) of whole plants over the mean dry weight of the nutrient Control ( $n=41$ ; 7.4 mg;  $p=0.05$ ). Therefore, the  $\alpha$ -anomer showed higher potency than the mixed anomers and these effective doses of 100  $\mu$ M MP and 1 mM TAM are compared to the nutrient Control in Figure 2.



**Figure 2.** Treatment of radish by 100  $\mu$ M pentaacetyl- $\alpha$ -D-mannopyranose (MP 100) and 1000  $\mu$ M tetraacetyl-D-mannopyranose, mixed  $\alpha/\beta$ -anomers (TAM 1000) with nutrients resulted in enhanced whole plant mean dry weight over that of the nutrient Control after 2 days. The  $\alpha$ -mannose was more potent than the mixed  $\alpha/\beta$ -anomers. Error bars indicate  $\pm$ SE.

Owing to enhanced growth and deeper pigmentation in response to treatments with mannosides, we sought a higher potency response, therefore, undertaking rapid radish assays with

methyl- $\alpha$ -D-mannoside. Soon after first morning light, exposure of radish sprouts to 500  $\mu$ M MeM resulted in notable greening of the cotyledon leaves within  $\sim$ 24 h. After 48 h, sprouted germlings treated to 25  $\mu$ M to 500  $\mu$ M MeM showed advanced growth responses as compared to Nutrient Control, roots and shoots showing robust enhancement of growth over the nutrient Control, as follow: Application of 500  $\mu$ M MeM to radish sprouts resulted in statistically significant 11% enhancement of mean dry weight (n=10; 10.3 mg) of whole plants over nutrient Control mean dry weight (n=10; 7.9 mg;  $p=0.000$ ). Treatment with 100  $\mu$ M MeM resulted in a highly significant 17% enhancement of mean dry weight (n=15; 10.9 mg) over the mean dry weight of the Nutrient Control (n=35; 8.7 mg;  $p=0.003$ ); 50  $\mu$ M MeM resulted in significant 11% enhancement of mean dry weight (n=10; 11 mg) over mean dry weight of the Nutrient Control (n=10; 9.9 mg;  $p=0.03$ ); and 25  $\mu$ M MeM resulted in a significant 12% enhancement of mean dry weight (n=20; 10 mg) over mean dry weight of the Nutrient Control (n=35; 8.7 mg;  $p=0.03$ ). Results of dosing radish roots with 25  $\mu$ M and 100  $\mu$ M MeM are graphically summarized in Figure 3.



**Figure 3.** Immersion of radish sprouts in methyl- $\alpha$ -D-mannoside (MeM) resulted in significantly increased whole plant mean dry weights of approximately 17% and 12% over the population of the Nutrient Control in 48 h. Error bars indicate  $\pm$ SE.

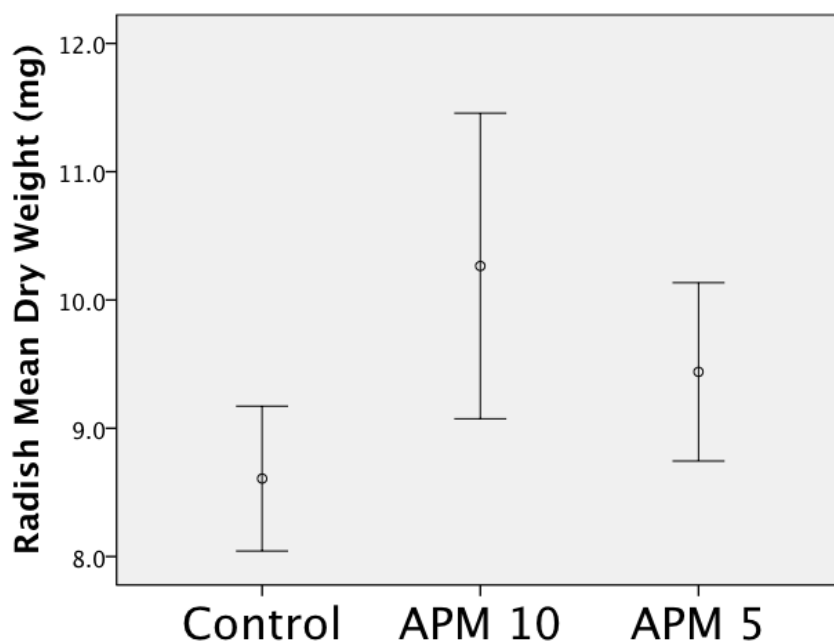


**Figure 4.** Treatment of radish with methyl- $\alpha$ -D-mannoside, right, showed enhanced pigmentation and general growth as compared to Nutrient Control, left. By harvest time on the 2<sup>nd</sup> day, expansion of treated cotyledon leaves and roots was clearly advanced over the Control. Scale bar = 1 cm.

Early on at 24 h, rapid responses were exemplified by visual comparisons of treated and control radish, shown in Figure 4. In one day, treatments with 500  $\mu$ M MeM, right, showed deeper pigmentation, longer roots and larger expansion of cotyledon leaves as compared to the Nutrient Control, left.

Experience with IG now guided the next experiments to test another arylmannoside, *p*-amino-phenyl- $\alpha$ -D-mannoside (APM), for higher potency than the aforementioned 25  $\mu$ M MeM. Immersion of radish sprouts in 100  $\mu$ M APM resulted in a statistically significant 10% increase of mean dry weight ( $n=10$ ; 11 mg) over the Nutrient Control ( $n=10$ ; 9.9 mg;  $p=0.01$ ). Hydroponic culture of radish sprouts on filter paper moistened with 10  $\mu$ M APM in nutrient solution resulted in a significant 13% increase of mean dry weight ( $n=20$ ; 10.3 mg) over Nutrient Control ( $n=40$ ; 8.7 mg;  $p=0.01$ ); but, the mean dry weight of 5  $\mu$ M APM ( $n=20$ ; 9.4 mg) was not significantly difference to that of the Nutrient Control ( $n=40$ ; 8.7 mg;  $p=0.06$ ). Results of the high potency response of radish roots to 10  $\mu$ M APM are summarized in Figure 5.





**Figure 5.** Treatment of radish sprouts in 10  $\mu\text{M}$  *p*-amino-phenyl- $\alpha$ -D-mannoside (APM 10) enhanced whole plant mean dry weight over the nutrient Control after 2 days. The population treated with 5  $\mu\text{M}$  APM showed no significant difference of mean dry weight as compared to the nutrient Control, but APM 10 showed the highest potency of the series, thus far. Error bars indicate  $\pm\text{SE}$ .

Representative selections from the population treated with an arylmannoside are compared to a nutrient Control, exhibited in Figure 6. A radish germling treated with 10  $\mu\text{M}$  APM, right, showed longer roots and larger expansion of cotyledon leaves as compared to the nutrient Control, left. Also, healthy root hairs are evident. In this experiment, we established the highest potency of the currently tested series of compounds and the growth responses that resulted may be attributable to the specific binding affinities of  $\alpha$ -mannosides to lectins.

**Glass Microbeads** The various  $\mu\text{Beads}$  that we tested provided support for hydroponic culture of plants with erect plants anchored by their roots in  $\mu\text{Beads}$  and detailed results were previously reported [16]. Aeration appeared to be adequate in our container cultures and we found that the larger the  $\mu\text{Beads}$ , the longer the durations of pH-stability. For example, 700  $\mu\text{m}$   $\mu\text{Beads}$  maintained neutrality for a full day or longer, but 100  $\mu\text{m}$   $\mu\text{Beads}$  rose above pH 8 within a few hours. When starting seeds in 700  $\mu\text{m}$   $\mu\text{Beads}$ , maintenance of moisture at the surface is critical to germination because the top layer tends to drain completely of water, possibly leaving the seeds to desiccate. At harvest, roots were immersed in full beakers of water, whereupon,  $\mu\text{Beads}$  rolled off of the roots and dropped to the bottom of the beaker. Cuttings of coleus propagated in 500  $\mu\text{m}$   $\mu\text{Beads}$  with daily exchanges of nutribead solution showed roots, intact hairs and caps within two weeks, as displayed in Figure 7.



**Figure 6.** Within 2 days, treatment of radish sprouts by 10  $\mu$ M *p*-amino-phenyl- $\alpha$ -D-mannoside, right, showed advanced growth as compared to Nutrient Control, left. Scale bar = 1 cm.



**Figure 7.** Root hairs of coleus after propagation in 500  $\mu\text{m}$   $\mu\text{Beads}$  are shown. A dip in water rolled  $\mu\text{Beads}$  off of roots, leaving the plant intact. The true color image, left, shows root hairs covering the top two-thirds of the white root; and the inverted color image, right, displays the root hairs in silhouette.

Paperwhite narcissus, was cultured in 700  $\mu\text{m}$   $\mu\text{Beads}$  in clear plastic 11 cm tall cylinders with <700  $\mu\text{m}$  diameter perforations for drainage. Roots and shoots are exhibited in Figure 8.

**Safety Handling**  $\mu\text{Beads}$  must be performed according to protocols that include reviews of Material Safety Data Sheets prior to experimentation. If spilled, these glass spheres are slippery underfoot. Therefore, spills must be picked up immediately with a vacuum cleaner. Bearing in mind that glass is over twice as dense as water, when lifting a full sack or a 20 L bucket of  $\mu\text{Beads}$ , take precautions to preserve healthy backs by requesting assistance. For laboratory utilization, sterilize  $\mu\text{Beads}$  separately from liquids, preferably by heating the dry glass in 200° C ovens overnight. Allow several hours for both  $\mu\text{Beads}$  and sterile aqueous solutions to cool to room temperature. Moisten  $\mu\text{Beads}$  only after cooling to <40° C to prevent bumping. Eruptions of wet  $\mu\text{Beads}$  in an autoclave may damage valves, controls, glassware, and instrumentation. Avoid touching  $\mu\text{Beads}$  to mucous membranes and eyes. Wear eye protection. Don a dust mask to prevent inhalation of  $\mu\text{Beads}$  and glass dust.

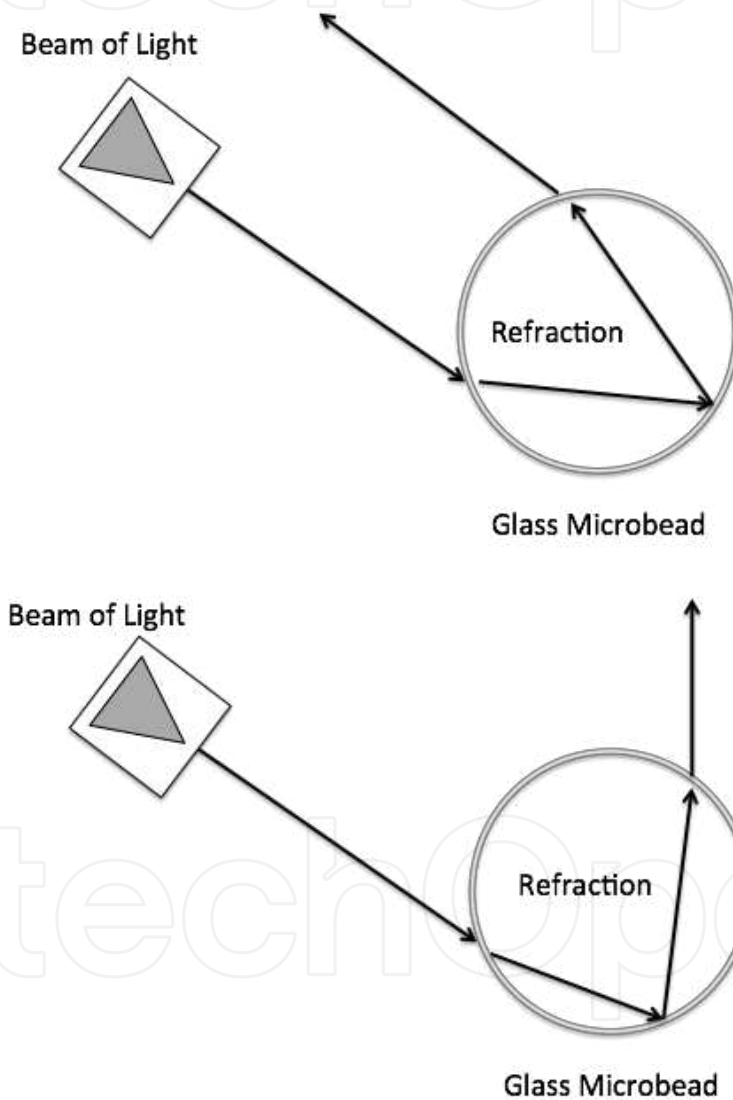
**Refractive Index** In kilns, glass beads are melted to form clear glass spheres with highly polished surfaces. Each  $\mu\text{Bead}$  is a micro-lens that refracts light. Moreover, diffuse reflection of light across the surface of a  $\mu\text{Bead}$  may send a fraction of the light in all directions. Light may be directed according to the index of refraction of the glass from which  $\mu\text{Beads}$  are manufactured. For example, a  $\mu\text{Bead}$  with a high index of refraction exhibits reflex reflectivity, sending light back toward its source. In contrast, a  $\mu\text{Bead}$  with a lower index of refraction may send a beam at a right angle to the incoming ray. In Figure 9, theoretical paths of light through a  $\mu\text{Bead}$  of high index of refraction, ~1.9, are compared to a  $\mu\text{Bead}$  with a lower index of refraction. Coincidentally, familiarity with reflex reflectivity at night from  $\mu\text{Bead}$ -coated road

markers and signs had been the primary barrier to consideration of utilization of  $\mu$ Beads in the light of the day, but the application improves solar illumination of plants. To be true, the diagram of Figure 9 is portrayed in two dimensions, but the refractive illuminatory effects of dispersed layers of  $\mu$ Beads are three-dimensional (3D). Solar illumination is diffuse and, therefore, a contiguous layer of  $\mu$ Beads refracts spherically in all directions. This 3D characteristic may be observed by viewing the phenomenon through a polarizing filter by which millions of refractions from  $\mu$ Beads spread over a 1-m<sup>2</sup> concrete pad may be seen as an aura

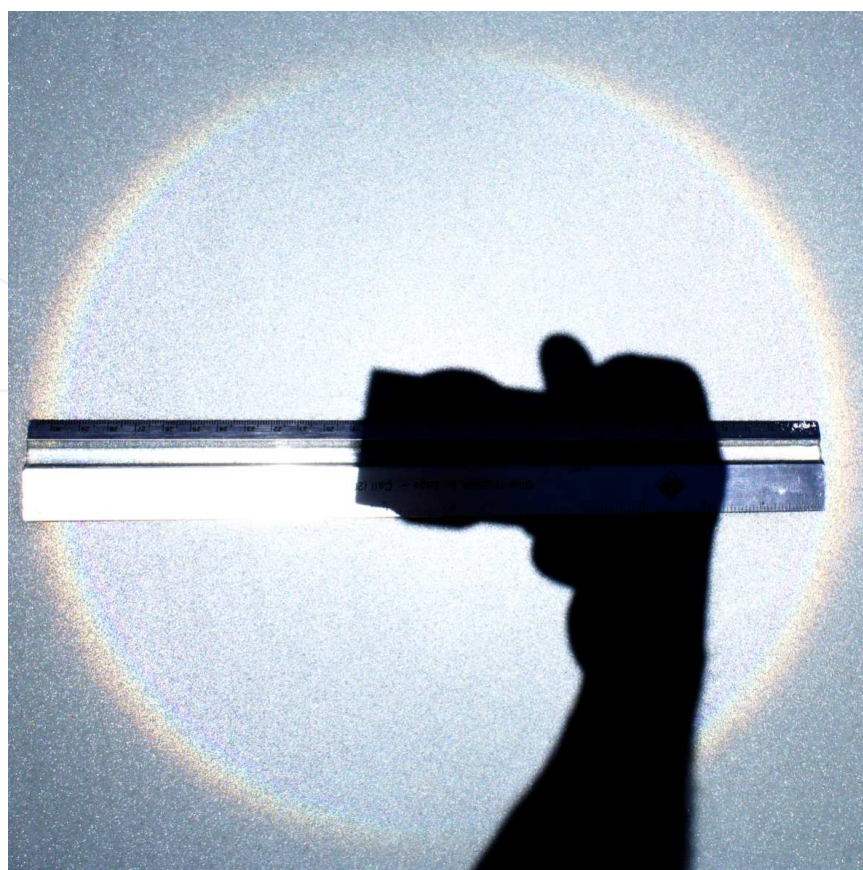


**Figure 8.** Cultivation of paperwhite narcissus in  $\mu$ Beads show a representative nutrient control, left, with a crown of roots up to ~5 cm in length around the basal plate; and, by comparison, when treated with IG, right, with roots elongated ~7 cm.

of a halo. The refraction of sunlight is exhibited in Figure 10 in which an aura surrounds the 16 mm wide-angle lens of the handheld camera at the center, 15-30 cm above the dome of light. Out of doors, measurements of intensities directly over substrates at 2.5 cm distance were as follow: Above sandy loam, 270 to 300  $\mu\text{Ein m}^{-2} \text{ s}^{-1}$  and over  $\mu\text{Beads}$ , 360 to 380  $\text{Ein m}^{-2} \text{ s}^{-1}$ ; sunlight refracted upward from the ground at approximately 20% higher light intensity than sandy loam. The additional light intensity from surface refraction may induce midday wilting for plants placed under direct sunlight and cultivated in  $\mu\text{Beads}$ , but may be corrected by preparing plants with applications of glycosides.



**Figure 9.** The index of refraction of  $\mu\text{Beads}$  determines the paths of beams of light. A  $\mu\text{Bead}$  with a high index of refraction, approximately 1.9, sends light back in the general direction of its source, top, a phenomenon known as reflex reflectivity. A  $\mu\text{Bead}$  with a lower index of refraction, approximately 1.5, may send light out at approximately a right angle to its approach, bottom. In each diagram, the symbol for a point source of light is a triangle in a box, labeled, "Beam of Light;" The circle labeled "Glass Microbead" represents a single  $\mu\text{Bead}$ ; and "Refraction" of a beam of light through the  $\mu\text{Bead}$  follows the direction of the linear black arrows. Under environments with diffuse lighting, a  $\mu\text{Bead}$  with a lower index of refraction may be a practical consideration.



**Figure 10.** An aura above a layer of  $\mu$ Beads is shown through polarizing filters. The spectral halo, best described as a three-dimensional rainbow of colors, was the result of upward projections of light by refraction through millions of  $\mu$ Beads spread in a 2 – 3 mm layer over a flat 1 m<sup>2</sup> level concrete area. A 30 cm ruler spans the diameter of the circle of light and the black silhouette is of the author's camera and forearm. The hemisphere is brightest toward its center; moreover, all points of the 1 m<sup>2</sup> covered with  $\mu$ Beads were approximately 20% higher in PAR intensity than adjacent surfaces.

#### 4. Discussion

Innovative glassware has been a hallmark of research in photosynthesis and the application of  $\mu$ Beads to refract light to the foliage emphasizes an integral role. At the start, we were faced with several problems; for example, the raw material source of  $\mu$ Beads, recycled sodalime glass, is alkaline. In practice, we found that the smaller the  $\mu$ Bead, the larger the relative surface area from which to extract native alkalinity; and their value in daylight had not been considered previously. As pH-stability became an important consideration, it became evident that the largest  $\mu$ Beads would be the preferred media for green plants. Treatment of  $\mu$ Beads with nutribead solution overcame the alkalinity problem while providing a buffered environment for cultivation. Continuous fertigation is a means of stabilizing the medium; and, ideally, automated pH controllers may be implemented to efficiently meter flow rates in a manner that permits high density planting. Such is the case exhibited in Figure 11, showing five paperwhite narcissus plants in a small container with their bulbs nearly appressed. As well, dense cultiva-

tion is applicable to protists as previously demonstrated on “Showa” [1] where frequent flow through of a pH-adjusted nutribead solution is matched by even drainage. Features of daylight enhancement are demonstrated in Figure 10, and because I was enhanced, application of  $\mu$ Beads to crops may entail broadcasting a thin 1-10 mm layer over the ground. As the index of refraction may be specified to direct light at different angles,  $\mu$ Beads of a lower index of refraction may be useful to start crops at subpolar latitudes during seasons for which the angle of solar illumination is low and bending light to a wider angle may distribute illumination advantageously. The application of  $\mu$ Beads in conjunction with glycoside formulations may be requisite to the continued growth of plants exposed to saturated-I, whether or not the overexposure is intentional. It is also important for this system of dual treatments with  $\mu$ Beads and glycoside formulations to maintain a soil at a pH that is amenable for growth. Clearly, for the cultivation of plants,  $\mu$ Beads may be of benefit significant enhancements of ambient light may be achieved by refraction through a multitude of glass spheres.



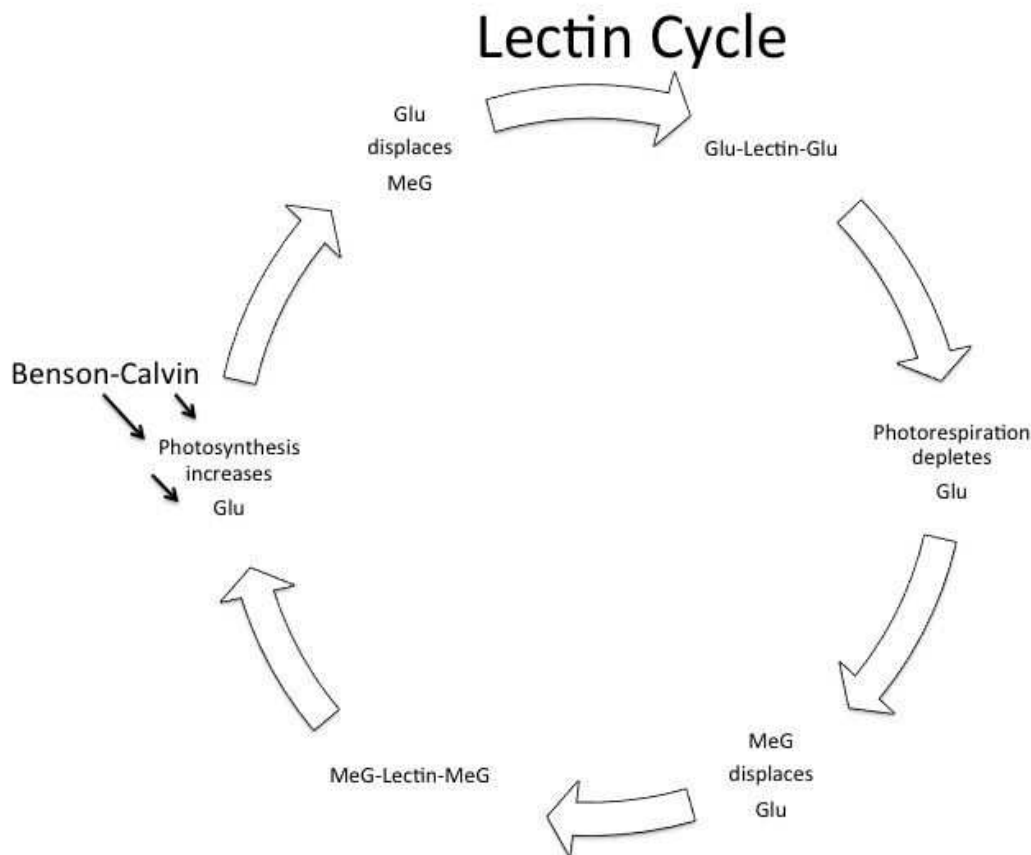
**Figure 11.** Five bulbs were planted in close proximity and the paperwhites blossomed while cultivated in 700  $\mu$ m  $\mu$ Beads. Roots showed through  $\mu$ Beads in the bottom half of the container. Colors from fluorescent illumination contributed to the blue and red hues of the moist  $\mu$ Beads that filled the container.

Applications of polyalkylglucopyranose to shoots of radish resulted in significant root enhancements over controls and, conversely, applications of polyacylglucopyranoses to roots of corn resulted in significant increases of shoots as compared to controls. Similar to findings of our previous experiments with  $C_1$  fragments and various glycosides [8,13,15], polyalkyl- and polyacylglucopyranoses required supplementation with nitrogen for significant improvements of growth. The production of ninhydrin-stained products may be from incorporation of nitrogen into protein, drawing attention to lectin as a protein complex from which stores of glucose could be displaced repeatedly by chemical competition with a glycoside. Lectin must be abundant and ubiquitous because, as we have found that Canola and corn respond to treatments of substituted glycosides, lectins occur in  $C_3$  and  $C_4$  plants. Moreover, not only do plant lectins bind  $\beta$ -glycoside, they bind preferentially to the  $\alpha$ -anomer. As much as a quarter of the protein content of seeds and up to ten percent of the protein content of leaves may be attributable to lectins; however, even with such abundance, the provenance of vacuolar lectins was that they served no endogenous role in plants [19,20]. Notably, plant lectins have structural requirements for specific divalent cations to bind sugars [21] and we are currently confirming these requirements with subtractive formulations of corresponding plant nutrient in conjunction with applications of glycosides to plants. The results of our current investigations are consistent with the highly specific binding affinities of mannosides to lectins, the corresponding potencies indicative of their tendencies toward proportionally higher orders of binding to lectins than for glucosides.

A case in point, the lectin from *Canavalia ensiformis*, concanavalin A (con A), specifies  $\alpha$ -tri-mannoside [23] and we have this core of complex glycans currently under examination. As the sequence of amino acids in this protein complex for recognition of mannosides is conserved in plants, the structural basis for specific recognition of mannosides in correspondence to the results of our experimental biology add compelling support of The Lectin Cycle. In conclusion, under conditions in which the cellular sugar concentration of a plant is diminished, chemical competition with substituted sugars may act to release other sugars from lectins—and this is an essential process to sustain viability. Consequently, binding affinities of lectins may service the natural displacements of sugars in periodic competitions, allowing energy to be reapportioned for growth resulting from metabolism of the freed sugars. For example, the concentration of methyl- $\beta$ -D-glucoside (MeG) remains nearly constant in the plant [22] and as a result of photorespiratory depletion of the concentration of glucose competition for binding to lectin by MeG arises and glucose is released. Then, under conditions more conducive to photosynthesis, critical concentrations of glucose are re-established to sufficiently high levels that a high concentration of glucose outcompetes the substituted glucoside. MeG is released and glucose wins a storage site on the lectin. To an extent, the timely and direct provision of free glucose may mitigate the effects of any impoverishment, whether by photorespiration, heat, drought, darkness, or other forms of stress that consume glucose faster than it is replenished by photosynthesis. The Lectin Cycle, schematically represented in Figure 12, may repeat many times in a day, releasing sugar from lectins at each lengthy photorespiratory event, followed by sugar refresh from the Benson-Calvin Cycle upon resuming photosynthesis. Indeed, Nature's response to major environmental stimuli by means of chemical competition is well known. For example, photosynthesis turns to



photorespiration as a result of oxygen outcompeting carbon dioxide for Rubisco. In a plant, the higher the quantity of lectins, the more capable it may be of storing and releasing sugars to endure prolonged depletion of glucose. This understanding led us to development of our rapid bioassay because we intended to exploit the high content of lectins in cotyledon leaves for the release of sugar. Additionally, when exogenous chemical competitors for binding sites on lectins are applied to plants, especially by the input of substrates that do not naturally occur in plants, such as *p*-amino-phenyl- $\alpha$ -D-mannoside, the duration of the effect may be substantially extended. Therefore, responses to treatments with glycosides must be carefully measured against the conformation of binding sites, biochemical structure, and their orders of preferences for prospective sugars. From another perspective, empirically formulated dosages of crops may possibly reflect the content and binding determinations of major lectins in a cultivar and our search in the future will be focused on the details of descriptions of the functions of substituted sugars in relation to defining suitability to lectins.



**Figure 12.** In The Lectin Cycle, various substrates displace glucose. The **Benson-Calvin** Cycle, left, contributes **Glu** (Glucose), of which, some is bound to lectins for storage, **Glu-Lectin-Glu**. Stress such as **Photorespiration depletes Glu**, right. Reduced concentrations of Glu create competition for lectin sites. For example, Methyl Glucoside (MeG) outcompetes Glu for binding sites as it is reduced to critically low concentrations. Thus, **MeG displaces Glu**, bottom right; and MeG binds to lectin, **MeG-Lectin-MeG**, bottom left. On return to photosynthesis, the **Benson-Calvin Cycle** once again contributes a sufficiency of glucose that raises the concentration of Glu to a competitive level and **Glu displaces MeG**, top left; thus, completing the Lectin Cycle.

## 5. Historical note

Steps toward management of the photosynthetic ecosystem were taken when coauthor Benson applied the first available  $^{14}\text{C}$  to plants [24, 25] and, most certainly, one of the great joys of life is to have made such extraordinary contributions early in the atomic era. For a time, Benson held the entire concentrated supply of  $^{14}\text{CO}_2$  because these were the most rare of all materials. Only the eminently prepared and bravest knew how to handle manmade atomic particles and this required the creation of equipment that had never been known before. For example, when Benson designed the “lollipop” to feed algae  $^{14}\text{CO}_2$  with even illumination [1] he developed a method for the “atomic culture” to quickly drop into methanol to stop the reactions at every step of The Path. The keys to the success of this apparatus were attributable to (1) flattening the glass vessel, thus creating an efficient photobioreactor; and (2) enlarging the bore of stopcock, permitting drainage of the entire volume in a second. An original apparatus is exhibited in Figure 13, showing the flat round face and the thin side view resembling a “lollipop” from which the glassware was so appropriately named. For this demonstration, the historically significant flat panel was filled with *Haematococcus thermalis* Lemmerman. Hence, we celebrate this 70<sup>th</sup> year of Benson’s originating concept and, felicitously, it is with best wishes that we also recognize his 50 years as a Member of the United States National Academy of Sciences [26] as well as his 78 years with the University of California; for, not only has the Path stood the test of time, Professor Emeritus Benson has, too!



**Figure 13.** The “lollipop” is a laboratory apparatus for the purpose of cultivating algae to track the path of  $^{14}\text{CO}_2$ . This first glass photobioreactor was designed by Andrew A. Benson and is exhibited to the left in face view, filled with *Haematococcus thermalis*. The large 4 mm bore of the stopcock is featured by fill with the green alga. Viewed from the side, right, the characteristic thin layer of the algal culture is revealed.

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