Technical

Hydroponic cultivation has high yield potential for TKS

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Executive summary

Rubber dandelion (*Taraxacum kok-saghyz*) produces natural rubber (cis-1,4,-polyisoprene), almost identical to rubber from the tropical rubber tree (*Hevea brasiliensis*), in root latex vessels (laticifers).

Cost-effective field production is not yet possible because of low rubber content, slow growth rates, poor competition with weeds and short season when crops are established by economical direct seeding instead of expensive transplants. Controlled environment vertical hydroponic systems have no weeds or dirt, are not subjected to outdoor productivity limitations and plants can reach a productive size in weeks, not months.

Rubber yields on a dry weight basis are not significantly different between field grown roots and hydroponic roots. Roots can be harvested repeatedly from the same plants. A scalable, modular vertical farming hydroponic system is being developed to match or exceed growth rates attainable in the current research system.

Natural rubber is ubiquitous in our economy, with more than 50,000 products requiring natural rubber, including military grade tires. Yet significant global shortfalls are projected (Warren-Thomas et al., 2015; Edwards et al., 2012).

Rubber dandelion (*Taraxacum kok-saghyz*, Rodin, TK) produces natural rubber (cis-1,4-polyisoprene) in root latex vessels (laticifers) almost identical to rubber from the tropical rubber tree (*Hevea brasiliensis*). However, cost-effective field production is not yet possible because of slow growth rates, weed pressure and a short growing season in the northern states (Cornish et al., 2016).

TECHNICAL NOTEBOOK
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Controlled environment hydroponic production in vertical farming systems provides a potential solution because there are no weed or dirt problems and they are not subjected to productivity limitations related to weather, day length or season. Plants can reach a size suitable for analysis and harvest in a fraction of the time required in fields.

Vertical farming is a method of growing crops in vertically stacked layers which takes up significantly less land than standard farming. It also allows the use of controlled-environment agriculture (CEA). CEA parameters can be controlled to optimize plant growth, including humidity, nutrients, temperature, air composition, and light spectrum and intensity. Some people view year-round vertical farming as the future of farming, in general, because of decreasing available land and the increasing need for food in urban landscapes. Additionally, vertical farming offers the benefit of year-round production.

Most of the alternate rubber efforts in the U.S. and Europe are focused on tire rubber. Although several companies (including Cooper, Goodyear, Bridgestone, Continental and Ling Long) have made various excellent tires from guayule and TK rubber, tire rubber is a commodity and its price cannot be matched using low yielding early stage germplasm grown on small acreages. Entry of premium rubbers into smaller volume, high performance niche markets, which can support higher raw material cost, are needed for commercial development. Vertical indoor farmed rubber can serve these premium markets.

It also is very important that the U.S. develop systems capable of rapid expansion in the short term, to protect its supplies of this critical raw material in the event of massive crop failure (disease or weather), or politically motivated interruptions of Southeast Asian supplies to the U.S.

4D scalable vertical farming systems could meet emergency military requirements much more quickly than existing field-based systems.

Methods

The custom-built automated hydroponics system used in this study was originally built by Crop King (Crop King, Lodi, Ohio), and later modified by T.R. Fontana, Wooster, Ohio (**Fig. 1**). The hydroponics system was comprised of eight separate aerated nutrient tanks, each feeding eight cylindrical wells for a total of 64 plant positions in a fully randomized design (**Fig. 1**). Each well had a diameter of 8 cm and a depth of 40 cm).

Flexible vinyl tubing (1.3 cm inside diameter, 1.6 cm outside diameter, blue color to inhibit algal growth) was used to drain and fill each tank with a specified nutrient solution. Drain and fill cycles were automated, cycling in pairs of tanks (1&5, 2&6, 3&7, 4&8). Each set was filled with nutrient solution for 2 minutes, in sequence, with additional aeration through ceramic stones located at the bottom of each well, with air provided by an electric pump.

Overflow tubes were set in each well to return excess solution to the appropriate tanks, maintain filled water levels and prevent overflow onto the table. After 2 minutes the tubes drained and roots were exposed to air for 6 minutes until the next refill cycle. During operation, the sides of the system were closed to inhibit algal growth.

In addition, the system was outfitted with automated pH monitors, which added hydrochloric acid as needed to maintain pH at 5.7 ± 0.3.

A modified, half strength, Hoagland's solution (Hoagland and Arnon, 1950) was used, except where otherwise stated, to grow *T. kok-saghyz* in the hydroponics system, as follows: reagent grade Haifa 4 mM L-1 $Ca(NO_3)_2$ and 4 mM L-1 KNO_3 (Haifa, Mata+m-Haifa, Israel); Innophos 1.3 mM L-1 KH_2PO_4 (Innophos, Cranbury Township, N.J.); Crop King 2.5 mM L-1 $MgSO_4$, 22.5 µM L-1 Fe, and a micro mix solution containing 0.09 µM L-1 Zn, 0.02 µM L-1 Mo, 0.075 µM L-1 Cu, 0.17 µM L-1 B, and 0.035 µM L-1 Mn.

The hydroponics table was placed in a greenhouse in Wooster, Ohio. Temperatures were maintained throughout the year with set points ranging from 18-21°C during the day and 15.5-21°C at night. During the winter, artificial light was provided for 10 hours from a 50-50 mix of 1,000-watt high-pressure sodium and 1,000-watt metal halide bulbs. In the summer, light was dependent on natural conditions with a shade applied to the greenhouse to reduce light penetration by 30 percent. Several experiments were performed to address specific research questions.

See **TKS**, *page 24*

The author

Katrina Cornish serves as endowed chair and Ohio research scholar for bioemergent materials, and also as director of the Program of Excellence in Natural Rubber Alternatives at Ohio State University.

Cornish

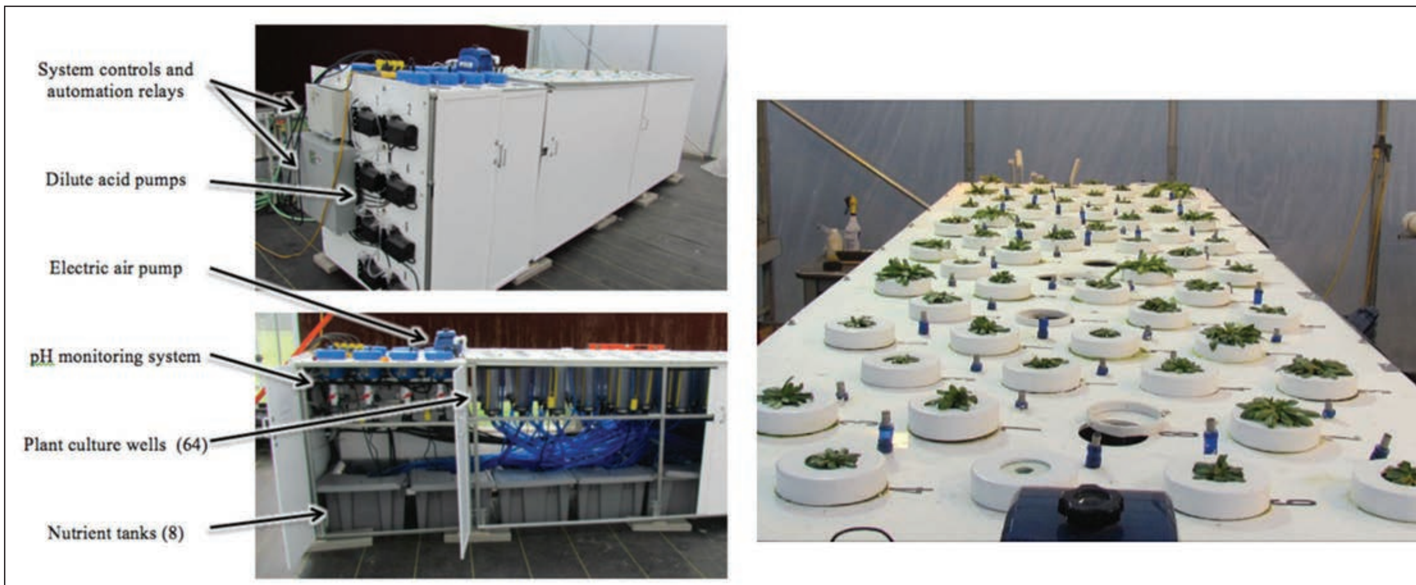
Cornish is an internationally recognized expert and innovator in alternative natural rubber/latex crops, their processing and formulation technologies and bioproducts, as well is in valorization of wastes.

Her accomplishments include being elected as a fellow of the National Academy of Inventors, as well as of the American Association for the Advancement of Sciences; 25 issued or pending patents, from OSU and her prior career; and 265 publications, numerous worldwide lectures and considerable public and private funding.

Cornish recently won a hat-trick of innovator awards: Ohio State University's 2018 Innovator of the Year, and 2019 Innovator of the Year for her college and for OSU's Institute of Materials Research.

She holds a bachelor's in biological sciences and a doctorate in plant biology, both from the University of Birmingham in Edgbaston, England.

Fig. 1: Research ebb and flow hydroponic system at Ohio State University. This system allows individual plants to be grown in an 8 x 8 fully randomized design.



Technical

TKS

Continued from page 23

Rubber concentration in field-grown and hydroponically grown roots:

Plants were direct seeded in autumn in high tunnel raised beds (Cornish et al. 2016). The following summer plants were harvested and 32 plants each with single and double taproot morphology were selected. Dandelions were washed and roots were cut 10 cm below the crown before random placement into the hydroponics system.

Half of each group were fed with half strength nutrient solution and the other half with one quarter strength. Dandelions were washed and cut 10 cm below the crown before placement into the hydroponics system, supported by Styrofoam rings and rock wool. Treatments were root number (plants with 1 or 2 roots) and nutrient solution strength (½ X and ¼ X), with plants of both root morphologies randomly assigned to specific locations across nutrient strengths.

Each individual well was tested daily to maintain nutrient solution conditions (pH of 5.7, 2 L of solution), and the dandelions were grown in the liquid media for four weeks.

Total fresh plant weights were recorded at intervals to analyze growth patterns, and after eight weeks, root and shoot weights, and root rubber concentrations were separately determined in the pre-existing 10 cm of root and in the new hydroponically grown roots. Rubber concentrations were determined using accelerated solvent extraction (e.g. Ramirez-Cadavid et al. 2018).

Rubber concentration and yield in original and regrown hydroponic roots:

Fig. 2: Average fresh weights of hydroponic dandelions measured throughout four weeks of growth in a liquid media. The ½ x Hoagland's solution (○) resulted in larger average growth than the ¼ x Hoagland's (◙).

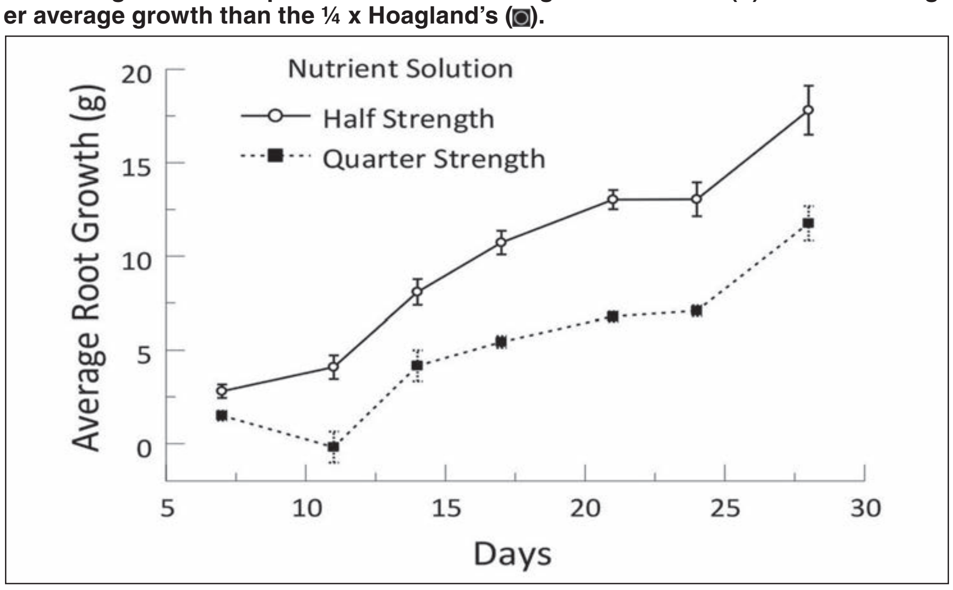


Fig. 4: Large (selected) and small dandelions (discarded) after 10 weeks of growth from seed.

A variety of TK dandelions were generated using root cuttings (Cornish et al 2016) and placed in the hydroponic system. After 10 weeks of hydroponic growth, 20 vigorous, hydroponically grown dandelions were sorted into eight groups based upon plant size and leaf morphology.

One plant within each grouping was designated as a control while the remaining dandelions harvested with a perpendicular cut to the long axis of basolateral root growth leaving 10 cm of root attached to the crown. The dandelions were replaced in the hydroponics system for eight weeks of additional growth.

Final growth measurements and rubber quantification, through ASE, were obtained using protocols developed for initial harvest methods. Data were analyzed by analyses of variance and significance claimed at $P < 0.05$.

Results

Rubber concentration in field-grown and hydroponically-grown roots:

During the first four weeks, plants grew significantly more rapidly in ½ strength than in ¼ strength nutrient solution (**Fig. 2**, P = 0.095). After eight weeks, the plants had clearly distinct roots types (**Fig. 3**). Also, it was clear that some plants grew much better than others (**Fig. 4** in hydroponics), and this also was the case in this experiment. Multiple two-way analyses of variance showed that the initial root morphology (one or two roots) did not significantly affect final root weight or rubber concentration, but the higher nutrient concentration did increase rubber concentration (P=0.019) (**Fig. 5**).

Fig. 3: Six-month-old rubber dandelions were dug from fields in Wooster, Ohio, then placed in a hydroponic system to produce new roots. Field grown roots (rosette and the white adventitious hydroponic roots, were harvested separately, and rubber content was quantified). In this plant, both root types contained 80 mg rubber/g dry root.

There were no significant differences between the rubber concentrations in the original roots and the hydroponically grown, more adventitious roots on identical plants (**Fig. 3**), indicating an over-arching genetic control pf rubber concentration. However, although it is clear that genotypic variation in size and root rubber concentration is large, some plants appear better suited to hydroponic rubber production than others (**Fig. 4**).

Current rubber germplasm is highly variable in these yield related parameters because TK is a diploid outcrossing species (like humans). Germplasm selections will have to be made to identify and propagate hydroponically adapted plants capable of producing high root rubber concentrations. The significant effect of nutrient concentration on rubber production indicates that rubber concentration can be maximized by optimizing a hydroponic production system.

Rubber concentration and yield in original and regrown hydroponic roots:

The demonstration that hydroponically grown roots produce similar rubber concentrations to field grown roots, opened the door to testing multiple root harvest potential in TK. After eight weeks post-root-cut, plants had regrown more root mass than they had accrued in the original 10 weeks of hydroponic production (**Figs. 6 and 7**), while rubber concentrations remained unchanged.

Discussion

The hydroponics research system proved highly effective at growing rubber dandelion roots containing rubber. The largest root system attained, under the same growing conditions, had a root fresh weight of 310 g, 30 g dry weight and, at 80 mg/g rubber, contained 2.4 g of rubber.

This indicates the potential of a TK hydroponic production system, which could contain millions of plants per acre, greatly exceeding the uncompetitive published rubber yields in TK field trials (Arias et al, 2016; Kreuzberger et al., 2016; Eggert et al., 2018).

However, the individual plant format of the research system reported here is poorly scalable. A new vertical farming system is needed which will be optimized to match or exceed the performance of the research system.

Ohio State University and American Sustainable Rubber Co. L.L.C. are collaborating to develop and optimize such a system. It has already been proven that rubber produced by soil-grown rubber dandelions is very similar to that from *Hevea brasiliensis* (Ikeda et al., 2016; Junkong et al., 2017).

However, very little is known about the quality of rubber produced from hydroponic roots, beyond its similarity in appearance to rubber from soil-grown plants. Thus, it is essential to fully characterize the rubber from repeated harvests of the same plants, especially among treatments and new germplasm genetically modified to produce enhanced rubber yields. In addition, TK roots produce latex in laticifers but these obviously cannot be tapped even if this were a cost-effective method. By harvest, much of the latex in field-grown roots has already coagulated (Abdul Ghaffar et al., 2016).

Solid rubber extraction methods must be used after drying to ensure that all latex is converted to the solid rubber form. Aqueous-based methods tend to retain non-rubber solids in the rubber fraction (Ramirez-Cadavid et al., 2017, 2018), failing the ASTM "dirt" standard set for *H. brasiliensis* rubber, although it is not yet known if slight contamination with lignocellulosic root debris is as damaging as actual dirt.

Solvent-based methods tend not to extract the high molecular gel, reducing yield (McMahan et al., 2015). However, it seems possible that healthy hydroponically grown roots, never subjected to environmental or edaphic stress, may retain a greater fraction of their rubber in the form of latex. If this proves to be the case, then the latex extraction process, originally developed for guayule and recently improved (Cornish, 2018), would likely be the most efficient method to extract TK rubber. TK latex can be coagulated post-extraction to serve solid rubber applications, as is done to 89 percent of *H. brasiliensis* latex post-tapping (Thomas, 2019).

Hydroponics also will allow TK nutrient requirements to be optimized to maximize root growth and rubber concentration. These optimized parameters should also be applicable to fertigation requirements in different field settings. However, the variability in diploid TK means that hydroponically adapted selections also will be needed.

Hydroponic technology and economic assessment for growing TK was conducted in 2017 by a collaborative student team project by the University of Akron College of Chemical Engineering and Ecole Nationale Superieure du Petrole et des Moteurs (IFP) School in France. (Report June 19, 2017, "Definition of a 100 tons/year pilot plant based on 3D [Hydroponic] Dandelion technology to produce natural rubber"). Depending on the variables, 1-5 ha of 4D TK would be required to produce 100 tons per year of TK rubber. Field grown dandelion have yet to exceed 0.1 t/y/ha.

Conclusions

Optimized vertical hydroponic farming, using 4D technology, integrating 3D indoor cultivation + 1D multiplication of harvest cycles from the same plants, indicates that TK rubber can be produced much more efficiently than in the field. This production system produces dirt and leaf-free rubber, which may be able to command a price premium in high value niche markets.

Acknowledgments

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Fig. 5: Comparison of rubber concentration in hydroponically grown roots, established from small transplants, before and after harvesting/regrowth (in pairs per individual plant). The treatments are (1) half and (2) quarter strength nutrient solution, whereas the root number groups describe the number of roots on the original transplant. The colors indicate the two bars belonging to each plant of the 32 shown.

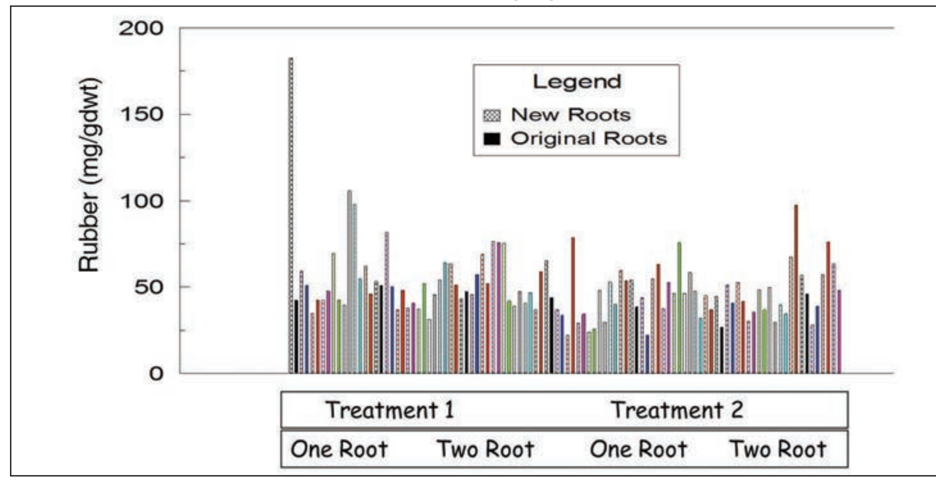


Fig. 6: Root masses at the start of the experiment (left panel) were harvested with a horizontal cut (center panel) removing adventitious roots below 10 cm. After eight weeks of continued growth in the hydroponics system (right panel), new adventitious root growth was analyzed. These photographs are the same plant.

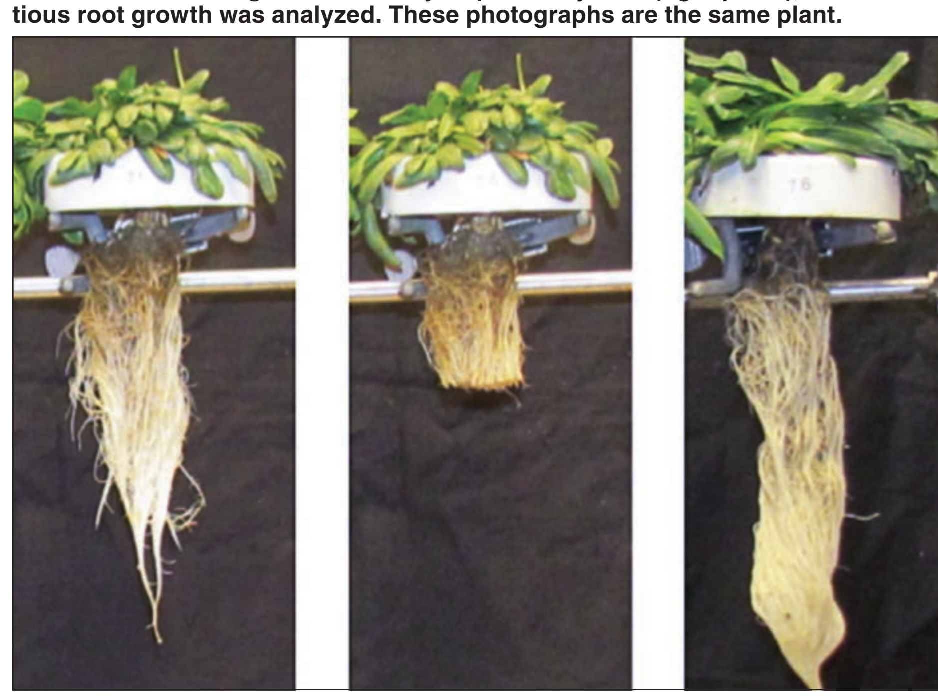
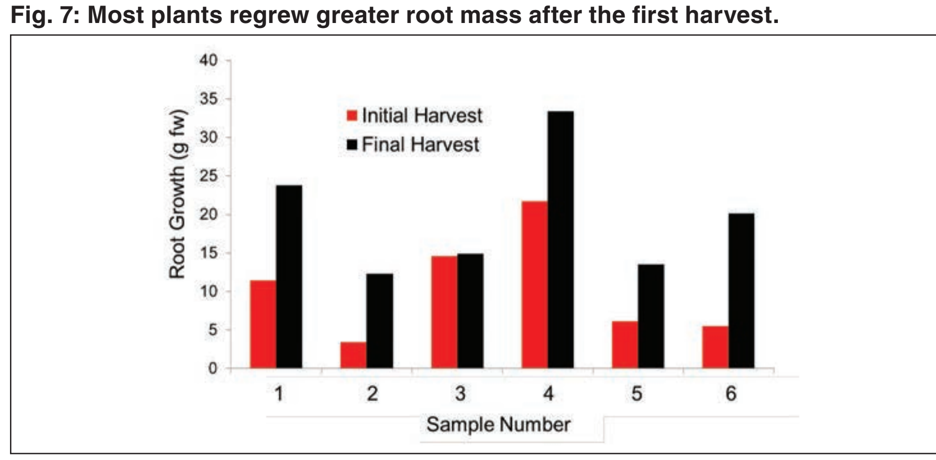


Fig. 7: Most plants regrew greater root mass after the first harvest.



Future technical notebooks

The technical notebooks in the next three issues of *Rubber & Plastics News* will include:

• Oct. 21: John Dick, *RPN* technical editor, paper comparing newest ASTM rubber tests to ISO TC45 rubber tests;

• Nov. 4: Greg Li, Dow Chemical Co., "Hight Heat Resistant EPDM Solution;" and

• Nov. 18: Sharon Wu, Dow, "Nordel EPDM Technical Solution for Automotive Dense Weatherstrip."

If you have a paper you would like to have considered for a future technical notebook, please contact Dick at john@rubberchemist.com.