Buckeye Gold storage

A study into rubber production in *Taraxacum kok-saghyz* with an emphasis on post-harvest storage

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Natural rubber is a vital resource for any developed country and is used in over 40,000 commercial products. By 2020 the USA may suffer a supply shortfall of 1.5 million metric tons of imported natural rubber.[1]

While the use of synthetic rubber has surpassed natural rubber in quantity, there are particular properties and high-performance applications that make natural rubber irreplaceable by synthetic rubber.[2] *Taraxacum kok-saghyz* (Buckeye Gold dandelion aka Russian dandelion, aka Kazak dandelion) is being investigated and developed as a domestic supplement or replacement of imported natural rubber from *Hevea brasiliensis* (Brazilian or para rubber tree) in commercial applications.[3] While Buckeye Gold rubber is of high quality, comparable to Hevea natural rubber, the species is not yet domesticated and the full production chain from seed to processing is still under development.

Since this new crop is being grown as an annual, it is expected that large quantities of the rubber-containing roots will be harvested and then stored for processing over the rest of the year to minimize the capital investment required for the processing plant(s) needed to extract and purify the rubber. Thus, post-harvest storage conditions must be validated for long-term storage.

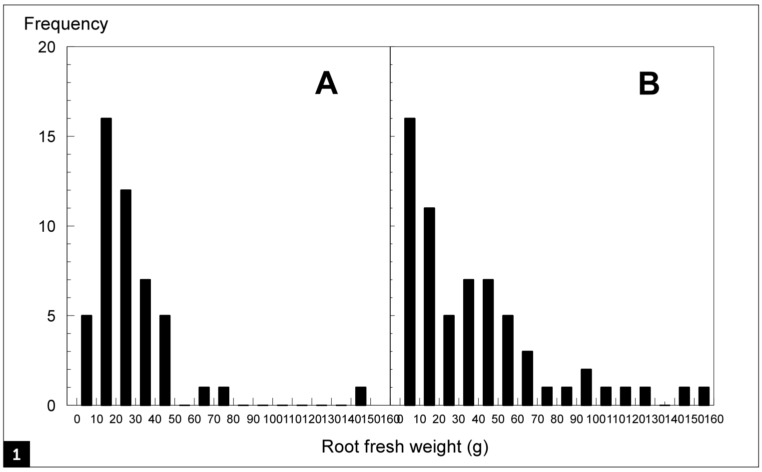

Figure 1: Root size distribution of roots from mixed lines harvested from high tunnels in (A) summer and (B) autumn of 2012

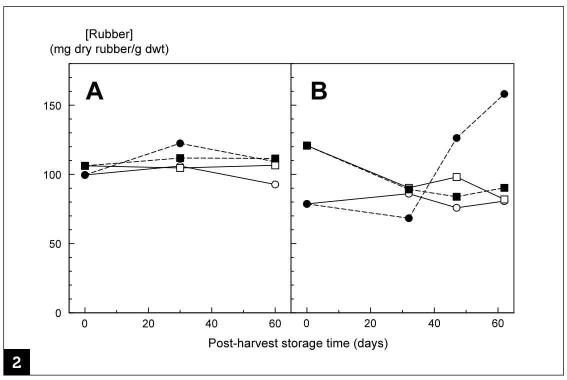

Figure 2: Rubber concentration (latex and residual rubber in the bagasse were combined) in fresh roots replanted in raised beds or stored at 4°C. Roots were harvested from a mixed line planting in high tunnels in the (A) summer and (B) autumn of 2012 and subdivided into two size classes at the beginning of each storage experiment. Large roots were replanted in raised beds —○—, or stored at 4°C --●--, (A) >7g, (B) >10g. Small roots were replanted in raised beds —□— or stored at 4°C --■-- (A) <7g, (B) <10g

We compared storage of fresh and dried roots, because Ohio has a humid summer climate and limited existing dry storage infrastructure, but does have considerable fresh fruit and vegetable storage facilities. In addition we investigated the impact of harvest time, storage temperature and root size on rubber yield.

Materials and methods

Two lines of Buckeye Gold were seeded into trays in a greenhouse on December 17, 2010 and transplanted into raised beds in high tunnels on February 6, 2011. The plants were harvested on June 8, 2011– the spring 2011 harvest of this study.

Buckeye Gold (mixed lines) was also direct seeded into raised beds in high tunnels. One set was seeded on November 29, 2011 and harvested between August 14, 2012 and August 28, 2012 – the summer harvest of this study. The other set was direct seeded on December 8, 2011 and harvested on November 2, 2012 – the autumn harvest of this study.

The plants from the summer and autumn 2012 harvests ranged in size from 6-253g fresh weight. Half were randomly selected, washed, weighed, placed in mesh bags and stored in a controlled, refrigerated (4-8°C) environment. The other half were replanted as controls into raised beds. Root weights were taken immediately before analysis on days 0, 32, 47 and 62 and ranged from 1-50g fresh weight. The roots harvested on Day 0 (n=9) ranged from 4-47g fresh weight. Three root systems were randomly selected from each treatment 0, 32, 47 and 62 days post-harvest. Latex, rubber and inulin were quantified using established methods.[4,5]

In another experiment, dry, chopped and pressed roots from a multiline planting were thoroughly mixed and 1kg samples were stored in the dark in either paper bags or plastic bags at three temperatures (4, 22 and 37°C). At each time point 6-10g subsamples were taken. About half this sample was finely ground and thoroughly mixed. Three 0.25g subsamples were then analyzed using accelerated solvent extraction[5], in which the samples were first extracted with acetone (non-rubber) and then with hexane (the rubber).

Results

The size distribution of the roots indicates that mixed Buckeye Gold lines vary widely (Figure 1). However, the peak size in the summer harvest of 10-20g fresh weight (Figure 1A) appeared to have grown to 30-50g by the autumn harvest (Figure 1B). At both times a minority of very large root systems was observed and some plants had root systems which remained very small.

Roots from the summer 2012 harvest, stored in the cold for 30 days, showed a higher rubber concentration than the control plants in roots of 7g fresh weight and above, but this level had declined by 60 days (Figure 2A). However, the control (replanted) roots also declined in rubber concentration between 30 and 60 days and so the cold treated roots >7g maintained a concentration much higher than the controls when stored refrigerated post-harvest. Cold storage had no effect on the rubber content of roots <7g fresh weight harvested in the summer (Figure 2A).

Changes in absolute rubber amount also were calculated (Figure 3) and we found that cold treatment did not increase the total amount of rubber in summer-harvested roots either below or above 7g fresh weight. This also proved to be the case when spring-harvested roots (Figure 4) were stored in the cold for 175 days.

However, the larger roots of >10g fresh weight, harvested in the autumn, responded strongly to cold treatment and doubled their rubber concentration in 60 days (Figure 2B). Over this time period the root fresh weight declined from 25.3 ±6.0g to 19.6 ±4.8g. The replanted control plants of >10g maintained a constant rubber concentration over the 60 days of the experiment, even though their mean fresh weight decreased from 25.3 ±6.0 to 20.3 ±4.6g (mean of 6) at 60 days. However, the small root systems of <10g did not increase rubber under cold storage and declined by 30% in rubber concentration in both treatments (Figure 2B). The average fresh weight of the control roots (from the replanted treatment) increased from 4.8 ±0.5 to 5.7 ±0.8g during the 60 days of the experiment. In contrast, the average fresh weight of the small roots in cold storage decreased from 4.8 ±0.5g to 3.0 ±1.1g over the 60 days of the experiment.

The changes in absolute rubber content confirmed that rubber biosynthesis was induced by cold treatment in roots >10g fresh weight but not in smaller roots.

We followed up on the cold-induction of rubber biosynthesis by separately analyzing latex, residual rubber and inulin content (Figure 6). It is clear that the concentration of inulin, a storage carbohydrate in Buckeye Gold roots, declines as the latex and rubber content increases. A clear lag was observed at 30 days of cold storage, indicating that the inulin is not immediately converted to rubber. Absolute changes also were calculated (Figure 7), confirming that rubber biosynthesis had been induced and that inulin content had declined.

Chopped, dry roots were stored at a water content of 10.2%. We observed that substantial fungal growth occurred on the roots stored in the paper bags at cold temperature but not on the roots stored in plastic bags. The water content of the roots stored in paper bags in the cold increased to 15-18% depending on the sample. The rubber content of the dry roots varied with the samples, but seemed less variable in the roots stored at 4°C than in those at the higher storage temperatures. The variation among samples is likely due to the variability in rubber content of individual root fragments and perhaps inadequate mixing of the materials before each sampling. Overall, the roots stored in plastic (Figure 8B) seemed to have lower rubber contents than those stored in paper (Figure 8A).

The random variation among samples was smoothed using a weighted average method (Figure 9). This approach does indicate that rubber content was lower when the roots were stored in plastic rather than paper. However, the amount of rubber was quite stable, overall, throughout the experiment with only minor variations. Interestingly, the microflora infesting the roots on the paper bags stored at 22°C and 37°C did not seem to degrade the rubber and were presumably living on the root inulin.

Discussion

It is estimated that every 50,000 acres of Buckeye Gold could create 4,500 jobs, and the USA could produce millions of acres of this industrial crop to support burgeoning global demand. However, for Buckeye Gold to become a viable addition to the supply of natural rubber from Hevea, the ability to produce and process rubber year-round must be achieved. This, in turn, means that harvested roots must be stored in large quantity to feed the processing plant, because snow cover in the farming region prevents root harvest during much of the winter. Saturation by snow melt also prevents root harvesting.

Cold storage can increase the rubber content in 30 to 60 days depending on harvest season, root size and storage condition, and can induce harvested roots to double their content of solid rubber and latex. It is perhaps not surprising that the rubber biosynthetic pathway is cold-induced, because similar cold induction is well-documented in the rubber-producing plant, guayule (*Parthenium argentatum*).[6,7]

Cold induction of rubber biosynthesis in Buckeye Gold roots is dependent on the size of the roots, with small roots (<10g fresh weight) unable to either perceive or translate the cold induction signal. Also, only the large autumn-harvested roots could be induced, summer-

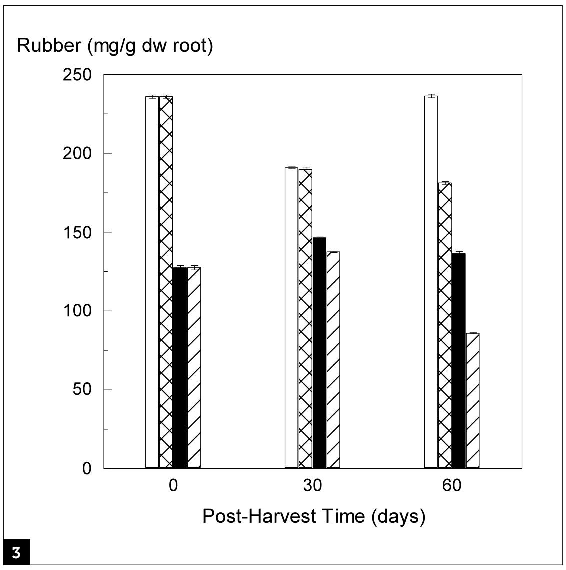

Figure 3: Rubber per root system (latex and residual rubber in the bagasse were combined) in fresh roots replanted in raised beds or stored at 4°C. Roots were harvested from a mixed line planting in high tunnels in summer 2012 and subdivided into two size classes at the beginning of the storage experiment. Large roots >7g fresh weight were replanted in raised beds ▭, or stored at 4°C ▨; small roots <7g fresh weight were replanted in raised beds ■ or stored at 4°C ▨

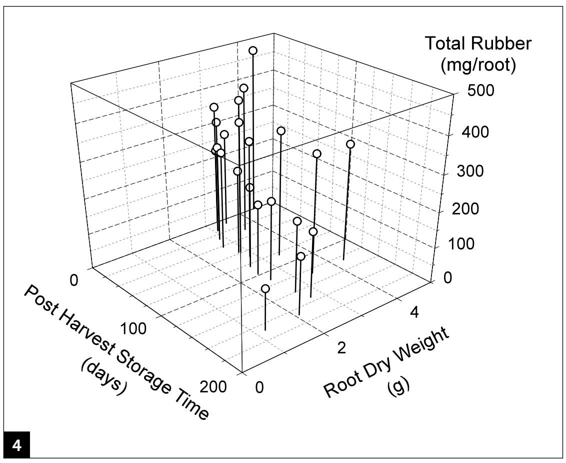

Figure 4: Total rubber per root system (latex and residual rubber in the bagasse were combined) in fresh roots harvested in spring 2011 and stored at 4°C plotted as a function of root dry weight and storage time. The fresh roots ranged from 75-80% water content

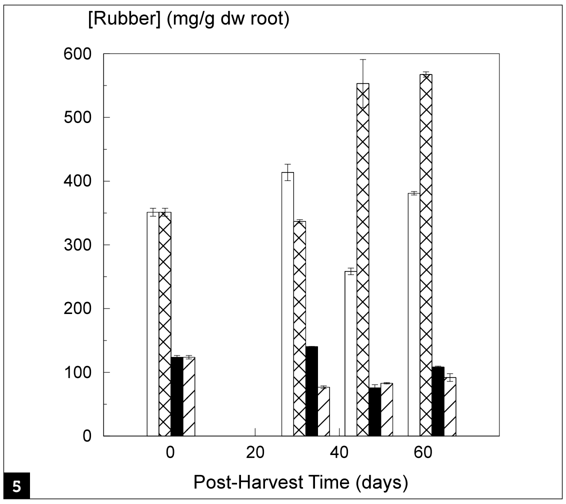

Figure 5: Rubber concentration (latex and residual rubber in the bagasse were combined) in fresh roots replanted in raised beds or stored at 4°C. Roots were harvested from a mixed line planting in high tunnels in autumn 2012 and subdivided into two size classes at the beginning of the storage experiment. Large roots > 10g fresh weight were replanted in raised beds, or stored at 4°C; small roots <10g fresh weight were replanted in raised beds or stored at 4°C

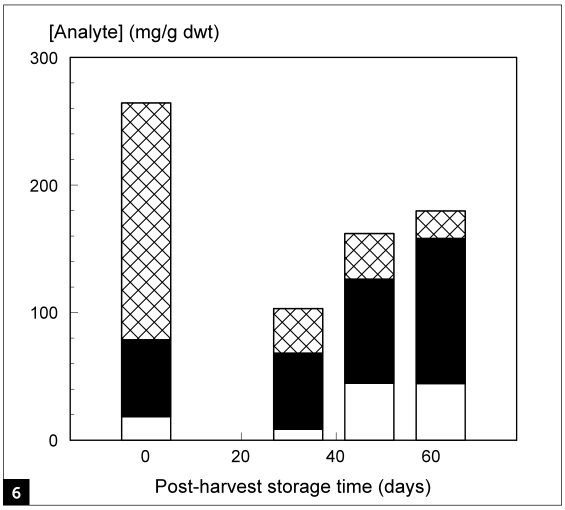

Figure 6: Concentration of latex, solid rubber and inulin; in fresh roots >10g stored at 4°C. Roots were harvested from a mixed line planting in high tunnels in the autumn of 2012. Each value is the mean of three

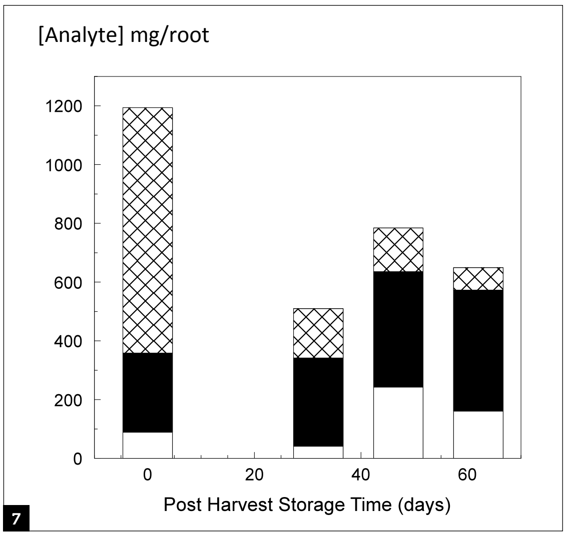

Figure 7: Total per root system of latex, solid rubber and inulin; in fresh roots >10g stored at 4°C. Roots were harvested from a mixed line planting in high tunnels in the autumn of 2012. Each value is the mean of three

harvested roots being unresponsive. The replanted roots >10g maintained rubber concentration but on average increased in size by about 5.8% dry weight over 62 days. This indicates that they also were synthesizing rubber and that the total rubber per root increased. However, the stored roots had no access to additional assimilate via photosynthesis and their rubber increase must therefore have been the result of metabolism of existing energy reserves.

The only notable energy reserve in these roots is the store of inulin and this must, therefore, be the source of substrates for the cold-induced rubber biosynthesis we observed in the larger roots in cold storage. In support of this hypothesis, inulin, a storage carbohydrate, rapidly decreased in cold storage in these roots, and there was a lag of several weeks as the simple sugars released from the degraded inulin were metabolized into substrates usable by the mevalonate[8] and rubber biosynthetic[9] pathways (Figures 6 and 7, between 30 and 45 days).

The wide variation in root size and rubber content among plants is a reflection of the early domestication stage of this plant. However, this variation suggests that classical selection and plant breeding approaches will rapidly reduce inter-plant variability and increase performance. It stands to reason that a combination of vigorous growth and high rubber concentration is likely to result in the best rubber yield per hectare. Cost analyses will need to be used to inform the processing protocols throughout the year. For example, except for autumn-harvested large roots, as discussed above, cold storage reduces the inulin content and weight of the roots while protecting the rubber, giving rise to roots with a higher rubber to biomass ratio than control roots. Whether the cost of storage is more than the savings in cost required to process these 'enriched' roots remains to be determined. Similar cost analyses will determine if even the large autumn-harvested roots should be cold-stored to increase the amount of rubber before processing.

Dry roots can be stored without much rubber loss for at least nine months and do not appear to required sophisticated storage conditions. Barn storage seems a likely low-cost option.

Conclusions

Post-harvest storage of rubber-containing roots will be necessary for rubber producers during the periods between harvests in order to enable rubber production facilities to work continuously. Dry roots can be stored for at least nine months without large losses, but the atmosphere in the storage container should remain dry. Fresh roots also can be stored refrigerated. Under these conditions, the rubber polymer is stable while the total weight of the roots declines. Large roots, above 10g fresh weight, when harvested in the autumn, increase their amount of rubber due to cold-induction of the rubber biosynthetic pathway. The roots degrade inulin to provide the substrates for rubber biosynthesis.

To maximize productivity, smaller roots should go to processing (or drying) immediately while larger roots can be stored to increase rubber yield and then dried at maximum rubber content. Introduction of Buckeye Gold into the natural rubber market would diversify the sources of natural rubber, adding protection to supply and prices for the international market, and reducing US dependence on imported natural rubber. Increasing the amount of rubber in Buckeye Gold roots post-harvest will accelerate the transition of the plant from an experimental rubber source to emergence in the natural rubber market.

Acknowledgements

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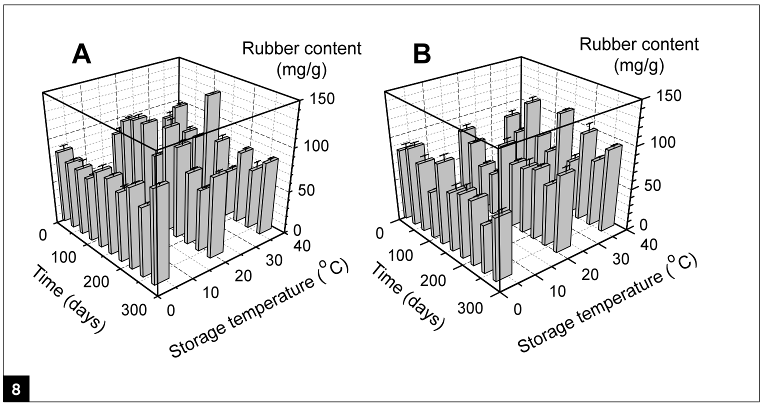

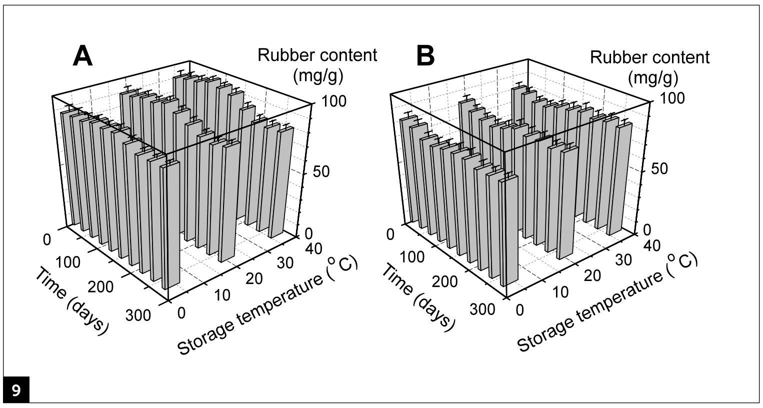

Rubber content in dry, chopped and pressed roots stored in the dark in either (A) paper bags or (B) plastic bags at three temperatures (4, 22 and 37°C). At each time point subsamples were analyzed using accelerated solvent extraction. Each value is the mean of three ± standard error **(Figure 8)** or the weighted average of three ± standard error **(Figure 9)**

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