Rubber Production under Cold Induction Treatment and Over-wintering Practice in *Taraxacum kok-saghyz* Roots

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ABSTRACT

Post-harvest cold treatment increased rubber concentration in *Taraxacum kok-saghyz* (TK), an alternative rubber crop. However, the effect of cold on rubber biosynthesis in growing plants is unclear. This study characterizes changes in cell structure, rubber particles ontogeny, and rubber yield during cold stress. Outside planting boxes and greenhouse hydroponic systems were established. Plants in planting boxes (covered and uncovered) were left to experience winter. Cold was applied to roots of hydroponically-grown plants using a chiller. Treatment effects were monitored by analyte quantification and microscopy after 25 and 50 days of cold. Cold treatment improved rubber yield in both planting systems after 50 days, except in the uncovered box where rubber biosynthesis was inhibited by freezing soil temperatures and snow. The cold also changed the cell ultrastructure and a new pathway for rubber particle ontogeny was observed. Overall, these studies provide useful information on the enhancement of rubber production in TK roots by cold temperatures, which may inform management strategies to enhance crop yields.

INTRODUCTION

Taraxacum kok-saghyz (TK) is being investigated as substitutes for *Hevea* (the rubber tree), because it produces high quality rubber and can grow in temperate climates [1]. Furthermore, TK rubber production can be influence by the environmental signals [2]. Cold temperature is an environmental stimulus involved in the induction of rubber biosynthesis in both guayule [2] and, post-harvest, in TK [3]. However, in our study, the main objective is to observe the cold temperature effects on rubber production while the plants are growing. Rubber is initially formed as particles in the cytosol and can be extracted in the form of a latex – an aqueous emulsion. When rubber particles coalesce or coagulated inside the roots they become solid rubber. These two forms together reflect the total rubber content of the root. Another objective is to microscopically characterize cold-induced changes in the ontogeny of rubber particles and cellular ultrastructure.

MATERIALS AND METHODS

TK plants were established and grown in outside planting boxes in soil, and in nutrient film hydroponic tables (NFT) in a greenhouse. Plants in boxes were mixed phenotypes, 6 and 12 months old in November 2016. The boxes were either left uncovered (Fig. 1a) to experience snow or were covered (Fig. 1b) with plastic. In hydroponic tables (Fig. 1c), clones of a single genotype were grown. After 4 months, cold temperature (10 ± 2°C) was imposed by nutrient medium chillers. Plants were harvested from both experiments after 25 and 50 days. and rubber contents were quantified, using accelerated solvent extraction (ASE) [4]. Latex content was also quantified) [5]. Histological samples of roots were fixed, dehydrated and resin-infiltrated before being sectioned, stained and viewed under a TEM microscope.

Figure 1: *Growth conditions (a) box, (b) covered box, (c) NFT hydroponic system.*

RESULTS AND DISCUSSION

Older roots generally had more latex and total rubber than younger ones (Figs 2 and 3). Also, after 50 days. higher latex contents were observed in roots form covered than uncovered boxed after 50 days (Fig. 2),suggesting that colder temperatures and snow cover inhibited rubber biosynthesis. The uncovered boxes had lower soil temperatures (2 ± 1°C) than the covered boxes (4 ± 1°C). Snow cover also places the plants into the dark, preventing even a low level of photosynthesis. Similar trends of age and cold treatment were observed in the hydroponically-grown roots (Fig. 3) even though only the roots were chilled and the temperature dropped to 10 ± 2°C. The cold treated plants had almost triple the rubber per root than controls, after 50 days. The minimal increase at 35 days but significant increase after 50 days parallels the response of roots cold stored post-harvest [3].

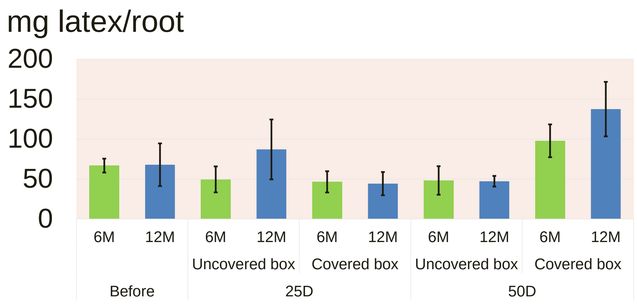


Figure 2. *Root latex content in plants grown in outdoor boxes for 25 and 50 days (means of 12 ± se).*

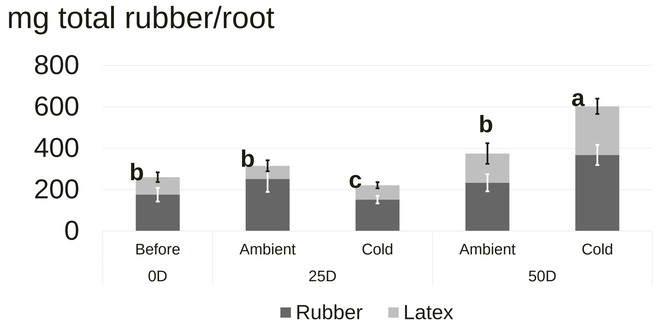
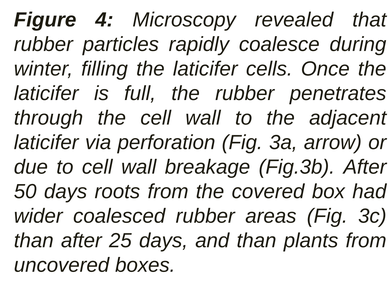
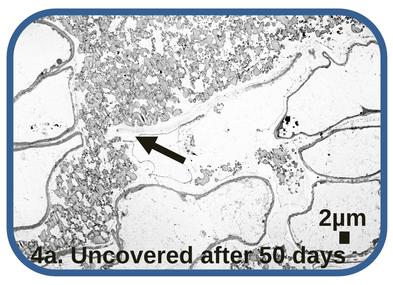


Figure 3: *Latex and rubber content in TK roots grown hydroponically (means of 12 ± se except for ambient/25D with 6 ± se) The same letter(s) are not significantly different at p<0.05 (LSD test).*

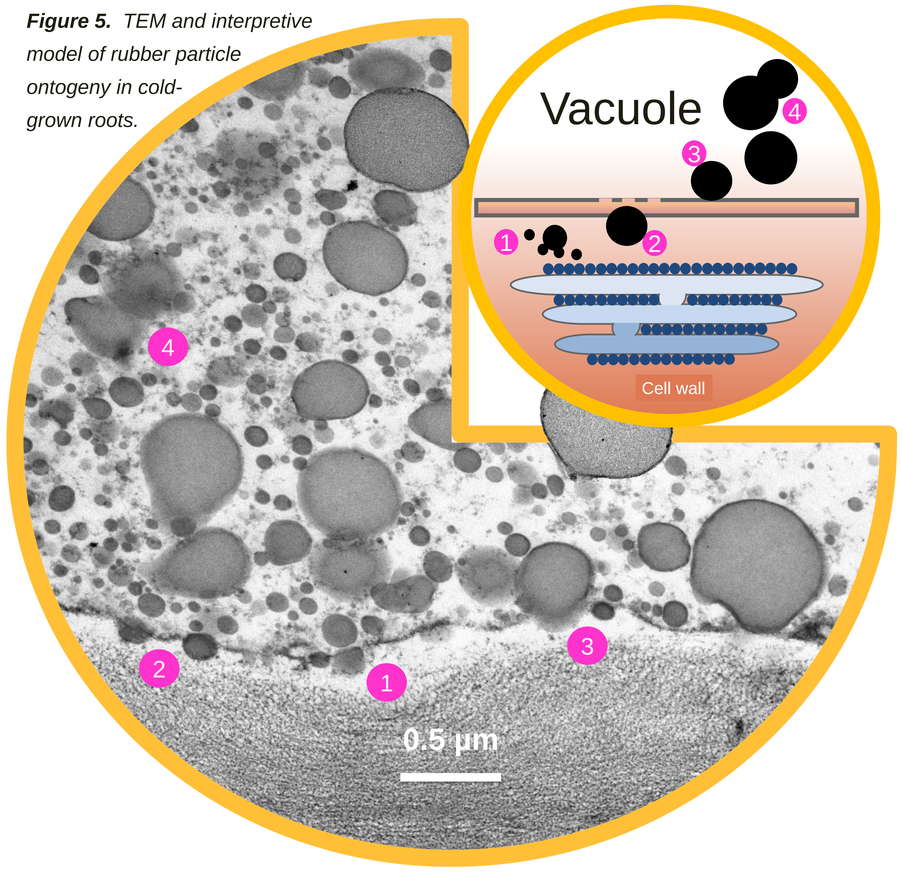
Figure 4: *Microscopy revealed that rubber particles rapidly coalesce during winter, filling the laticifer cells. Once the laticifer is full, the rubber penetrates through the cell wall to the adjacent laticifer via perforation (Fig. 3a, arrow) or due to cell wall breakage (Fig.3b). After 50 days roots from the covered box had wider coalesced rubber areas (Fig. 3c) than after 25 days, and than plants from uncovered boxes.*





Another rubber particle ontogeny pathway was discovered in cold-grown roots. These rubber particles appear to be produced from compressed layers of endoplasmic reticulum located very close to thick cell walls, without participation of Golgi apparatus as occurs in ambient temperatures (Fig.5). However, rubber particle development is similar to vesicular rubber particle production. It begins with (1) the accumulation of rubber globules to form a rubber particle; (2) the rubber particle moves near the tonoplast; (3) the rubber particle is ejected into the vacuole; (4) in the vacuole, the rubber particles coalesce with other rubber particles and increase in size.

Figure 5. *TEM and interpretive model of rubber particle ontogeny in cold-grown roots.*



CONCLUSIONS

Cold temperatures enhance rubber production in intact living plants to a similar extent and over a similar time frame to cold induction of rubber biosynthesis in harvested stored roots. Severe exposure prevented induction. Rubber particle ontogeny in the cold differs from particle formation at ambient temperatures, because Golgi bodies seem to not be involved and particles coalesce to a much greater extent leading to very large vacuolar particles in cold grown roots.

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ACKNOWLEDGEMENTS

We thank Karolyne Kaszas (Molecular and Cellular Imaging Center, OSU/OARDC) and MRB for sponsoring the student. Funding was provided by the PENRA Consortium, Ohio Third Frontier, OARDC, and USDA NIFA, Hatch project 230837