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# Optimal Planting Density of *Taraxacum kok-saghyz* Bred for Large Root Size: Seed, Latex, and Rubber Yields

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***Abstract.*** **The rubber dandelion, *Taraxacum kok-saghyz* (TK), produces similar quality natural rubber (NR) to *Hevea* but can be grown in temperate regions and could supplement the global supply chain, providing NR security to countries relying on imports. This study examines the effect of planting density on yield parameters uniquely using an advanced rubber dandelion accession bred for large root size in the field for eight generations. Plants were grown at densities of 0.31, 0.62, 1.23, and 2.47 million plants/ha in greenhouse planting boxes. The effects of planting density on plant growth, seed set, latex, and NR yields were compared with previous literature where seed from the US Department of Agriculture 2008 TK collection from Kazakhstan or from interbred plants of that collection were planted at different densities. Maximum seed production in the greenhouse environment was equivalent to 219 million seed per hectare. Latex concentration in processed roots ranged from 5.3 to 9.9 mg/g dry root. The weight average molecular weight of rubber from this investigation was sufficient for rubber product manufacturing (1255 to 1744 kg/mol) and did not differ significantly at different planting densities. A maximum rubber yield of 60 kg/ha in 4-month-old plants was attained at a density of 1.23 million plants/ha. This yield was lower than a previous report yet higher than several others and was therefore compared with all other TK planting density studies to assess the key factors that affect rubber yields in addition to planting density itself. High rubber yields were supported by high planting density, longer growing season, and late harvest in a single season. Optimal planting density must be determined for each distinct population, because advanced larger-rooted lines should be wider spaced than wild-type TK populations.**

Natural rubber (NR) is used in tens of thousands of products and is relied upon globally for industrial, medical, and transportation devices. Over 70% of the NR produced is used in tires upon which the world depends for transportation ensuring the efficient flow of goods and services to support national economies. The United States deemed NR a critical raw material for national security and the domestic economy in the Critical Agriculture Materials Act of 1984. The European Union similarly considers NR to be a critical raw material (European Commission 2020). Although approximately 2500 plant species produce NR, they do not all produce rubber of high quality and molecular weight like the tropical rubber tree, *Hevea brasiliensis*. *Hevea* trees grown as clonal scions on seedling root stocks continue to be the predominant source of NR globally. Also, >90% of NR is produced from a limited geographic region of Southeast Asia that cannot expand without illegal deforestation of tropical rainforests. Most of the world must import NR, their economies depending on the survival of threatened rubber tree plantations and the continued functioning of global supply chains.

*Taraxacum kok-saghyz* (TK) is an alternative rubber-producing species with potential to supplement the NR supply chain. Unlike the tropical rubber tree, TK can grow in temperate climates, making it possible to grow in many more places than *Hevea*, including the northern United States and Europe. Best growing practices for TK are still being established, with the species undergoing domestication in several countries by research groups and companies with commercial interests (Böttner et al. 2023; Continental Tires 2018; Feng et al. 2021, 2023; Goodyear Tire Rubber Co. 2022; Inoue et al. 2019; Liu et al. 2024; Ohio State News 2024). One of the practical questions not yet answered regarding TK production is how close together the plants should be spaced for optimal seed, root growth, latex, and rubber yield. While the question of TK's potential dry rubber yield per hectare has been previously explored, planting densities studied were mostly very low (0.08 to 0.5 million plants/ha), insufficient for commercialization (Arias et al. 2016; Eggert et al. 2018; Kreuzberger et al. 2016). In a planting density study in which higher densities were used, much higher amounts of rubber were reported (Bates et al. 2019). These various studies all used seed obtained from the US Department of Agriculture (USDA) TK collections (Hellier 2011) or from progeny of plants grown from that collection and interbred. However, new populations have been developed at the Ohio State University (OSU) through multiple rounds of selections for large root size in the field. The most advanced populations (eight rounds of selection) are much larger than their original progenitors, which could greatly affect their optimal planting density.

This study also investigated bulk seed production in a greenhouse environment for the first time, outlining pollination, seed collection, and cleaning techniques. These methods will be important if TK is commercialized and produced at a large scale, requiring millions of seeds per hectare.

In addition, highly effective biological methods to control insect pests in TK greenhouses are described, completely eliminating the use of pesticides. Finally, unlike all previous planting density studies, the latex fraction was extracted from roots, purified, and characterized using size-exclusion chromatography to determine whether planting density affected rubber molecular weight and/or polydispersity.

## Materials and Methods

*Chemicals, reagents, and insects.* The reagents used in the rubber extraction solution for homogenization were KOH (Ward's Science, Rochester, NY, USA) and $Na_2SO_3$ (Amresco, Cleveland, OH, USA). This alkaline extraction solution was composed of 0.2% (m/v) KOH and 0.1% (m/v) $Na_2SO_3$. Glacial acetic acid (Thermo Fisher Scientific, Waltham, MA, USA) was used in latex quantification. Carboxymethyl cellulose (CMC) (MP Biomedicals, Solon, OH, USA) and ethylenediaminetetraacetic acid (EDTA) (Fisher Scientific, Waltham, MA, USA) were used in latex purification. Tetrahydrofuran (THF) (containing 0.025% butylated hydroxytoluene as a preservative) (Fisher Chemical, Waltham, MA, USA) and a polystyrene standard ($M_n$ 30 kg/mol, Đ 1.05; Pressure Chemical, Pittsburgh, PA, USA) were used in size-exclusion chromatography (SEC) analysis. Biological pest controls and a medium-sized beehive of bumblebees were ordered from IPM Laboratories, Inc. (Locke, NY, USA).

*Seedling production.* The TK population India is the product of eight rounds of breeding selection for large root biomass in the field from population Alpha, progeny of the 18 original TK germplasm collections by USDA scientists in Kazakhstan (Hellier 2011) and interbred by open pollination at OSU. To create each generation, 50 of the largest plants were selected. When population India was generated, these 50 plants all had root fresh weights greater than 14 g and then were interbred to generate the India generation. Roots of India plants are 1000% larger, on average, than roots of the original Alpha population (Fresnedo-Ramirez J, unpublished observation). TK needs high humidity after seeding (for roughly the first month) to thrive, making direct seeding challenging and transplanting the preferred method for establishing plants in the greenhouse (GH). Transplants also allow specific planting densities to be established.

India seed for GH production were sown into 200-cell flats of Promix FPX biostimulant medium in Oct 2023. Seedlings were germinated in a temperature-controlled, plastic-walled greenhouse (160.54 $m^2$) at the Ohio Agricultural Research and Development Center in Wooster, OH, USA (lat. 40°46′20.9″N, long. 81.93°55′19.4″W). Average temperature, *PAR*, and daily light integral in the GH were 18.4 ± 1.1 °C, 452.0 ± 57.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 19.5 ± 2.5 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. Supplemental lighting from metal halide bulbs was provided on cloudy/low PAR days. Plug medium was kept extremely moist with daily misting for the first week until radical cracking and cotyledon emergence, at which point watering was typically every other day. Plugs were rewatered to saturation before they could completely dry out.

*Greenhouse production.* In Nov 2023, at the six true leaf stage (4 weeks old), seedling plugs were transplanted into 20 raised planting boxes (beds) (1.83-m length × 1.22-m width × 0.76-m depth) containing moist Promix BX mycorrhizae medium in the same GH, followed by overhead watering. The plants were arranged in six rows 20 cm apart with an irrigation dripline placed between each row (five per bed). Each dripline was equipped with an individual valve that allowed for control of watering. Driplines in the beds were turned on for 3-h intervals as needed to maintain moisture in the bottom third of the bed (every 1 to 2 weeks). In-row spacing is described under "Planting densities."

*Planting densities.* Previous work in outdoor planting boxes estimated that the optimal planting density to maximize TK root size and rubber yield was between 2.47 and 4.94 million plants per hectare (Bates et al. 2019), using less advanced TK Alpha germplasm with much smaller roots and rosettes on average than the currently available India population used in our study.

Four planting densities were tested (Table 1). The lowest planting density (125,000 plants per acre or 31 plants/$m^2$) is the standard density used for population seed increases in breeding selection of TK at OSU and is intended to give the plants plenty of room to grow, flower, and set seed. It is not well suited to commercial-scale production. The initial density was doubled three times up to a maximum planting density of 1 million plants per acre or 247 plants/$m^2$, generating the four different densities planted. The two highest densities matched the two lowest densities used previously (Bates et al. 2019). Because India's plant size is several times larger than Alpha's, the density of 247 plants/$m^2$ was expected to cause significant crowding of the plants, and so the two lower densities were included. The lowest density also is similar to the highest densities used in the related literature (Arias et al. 2016; Eggert et al. 2018; Kreuzberger et al. 2016).

Each planting density was replicated across five beds. All planting densities and replicates were randomly assigned to the 20 beds using Microsoft Excel randomization. Beds with planting densities of 31, 62, 124, and 247 plants/$m^2$ had 11, 23, 46, and 92 plants in each of their six rows, respectively.

*Greenhouse pest management.* Numbered yellow sticky cards were arranged at plant height in beds at the front, center, and rear of the GH to assess potential insect pest developments and their location in the GH. Sticky cards were checked for pests, replaced, and beds were randomly scouted on a weekly basis. As pests appeared or were anticipated based on pest arrival times during past grows, beneficial insects were applied. Generally, *Amblyseius cucumeris* mites in sachets at a rate of 155.7 insects/$m^2$ were used to control thrips, *Aphidius colemani* aphid parasitic wasps in bottles at a rate of 3.11 insects/$m^2$ were used to control aphids, a 50/50 mix of *Encarsia formosa* and *Eretmocerus eremicus* parasitic wasps on cards at a rate of 62.27 insects/$m^2$ was used to control whiteflies, *Amblyseius swirskii* mites in sachets at a rate of 155.7 insects/$m^2$ were used to control thrips and whiteflies, and *Chrysoperla rufilabris* green lacewings, as both eggs and larvae, were applied at a rate of 31.1 to 62.3 insects/$m^2$ to control aphids. *Hippodamia convergens* convergent lady beetles, from bottles at a rate of 28.02 insects/$m^2$, were also used as curative control agents for aphids. Beneficial insects were typically added to the GH every 2 to 3 weeks to manage pests until the last month of production, when the only pest remaining in large numbers was thrips, which were not likely to kill the plants before harvest. The plants were grown entirely using biological pest controls because at no point in the production cycle did pests become so numerous that pesticide spraying was necessary. This was the first time an entire TK greenhouse growth cycle was managed with only biological controls at OSU.

*Pollination and seed collection.* TK is a predominantly outcrossing species, and most plants will not develop seed without cross-pollination with pollen from other TK plants. The first flower buds began forming at week 6 in the GH beds (10 weeks old). Plants were open pollinated with commercial bumblebees (under "Chemicals, reagents, and insects"), which effectively pollinated the entire planting area as they traveled among the flowers. At 12 weeks old, most beds had open flowers that had been pollinated, closed, and then reopened displaying typical white dandelion seedheads, which contain seeds each individually attached to a pappus that allows them to be wind dispersed over considerable distances (Cummins et al. 2018; Tackenberg et al. 2003).

Seed was collected thrice weekly from 24 Jan to 5 Mar using a shop vacuum to suck the ripe seed from each bed. Inside the vacuum,

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Table 1. Planting densities and the number of plants per bed assigned to each treatment.

| No. of plants per bed | In row plant spacing (cm) | Planting density (plants/acre) | Planting density (plants/m$^2$) | Planting density (million plants/ha) |
|---|---|---|---|---|
| 69 | 8 | 125,000 | 31 | 0.31 |
| 138 | 4 | 250,000 | 62 | 0.62 |
| 276 | 2 | 500,000 | 124 | 1.23 |
| 552 | 1 | 1,000,000 | 247 | 2.47 |

cloth bags were rubber banded around the inlet tube to catch all seeds, pappi, dirt, plant matter, etc., as they were vacuumed from flowers. Seed from cloth bags were then transferred to bed-specific paper bags.

*Harvest and root homogenization.* The plants were harvested from the beds when 4 months old in Mar 2024. At harvest, rosettes were cut from the crowns of plants and roots, and the rosettes were counted to determine the number of surviving plants from the initial planting densities. The total fresh weight (fwt) for the rosettes and roots of each bed were measured the day of harvest. The rosettes were then set out evenly across their beds to dry in the sunny GH. The rosettes were completely dry after 3 weeks and were reweighed for a dry weight (dwt). Roots were thoroughly washed the day of harvest and then cold stored at 6 °C in a refrigerated vegetable storage cold room.

Roots from each bed were removed from storage and homogenized 1 month after harvest using previously published methods (King-Smith et al. 2022). Roots were weighed (fresh), homogenized in a latex extraction solution [0.2% (m/v) KOH and 0.1% (m/v) $Na_2SO_3$] at a root fwt:rubber solution ratio of 1:1.33 (w/v). The root slurry from homogenization was then filtered through 0.1-mm Miracloth mesh filter screens (Illusions Screenprinting, Wooster, OH, USA) and pressed through a custom-made hydraulic press (Pearson et al. 2013) to obtain the liquid filtrate containing extracted rubber particles (latex phase). The retained solid bagasse cake containing root skins and other pieces of plant matter was dried at 50 °C and stored at room temperature until analyzed for residual dry rubber (Section 2.10). The pH of the liquid filtrate was adjusted to 10 to 11 using KOH pellets and checked daily to maintain pH above 10 at all times until centrifugation. Total extracted filtrate volume was recorded for each bed. Three 1-mL aliquots were taken for latex quantification (LQ), and three 1-mL aliquots were taken for homogenate solid quantification, which allowed calculation of latex concentration and dry root weights. After LQ, all 20 filtrates were adjusted to 20 mM EDTA to enhance the amount of extractable latex (King-Smith et al. 2023). After individual LQs were performed for each bed, the 20 beds were pooled by planting density into four buckets for latex extraction and purification.

The total bed root dry weight was calculated using the average dry solids concentration in the liquid homogenate multiplied by the total homogenate volume to determine dry root weight in the homogenate. This value was added to the weight of the solid bagasse cake retained in the hydraulic press to calculate a total bed root dry weight. The average dry weight of a single root system was then found by dividing the total bed root dry weight by the number of root systems harvested from that bed.

*Latex extraction, purification, and quantification.* Latex was extracted from the pooled root filtrates via centrifugation followed by creaming agent assisted separation, as previously described (King-Smith et al. 2022). A Beckman J2 centrifuge with a fixed angle rotor (model JA-14) was used to spin filtrates at 15,300 $g_n$ (10,000 rpm) for 20 min at 20 °C. After spinning, latex floated to the top of the bottles and was vacuum pipetted off the top layer as crude latex.

The crude latex was then suspended with an equal volume of the KOH + $Na_2SO_3$ latex extraction solution adjusted to 0.2% CMC. The mixture was then suspended in combinatorial chemistry vessels without frits (Wilmad-LabGlass, Vineland, NJ, USA). Subnatant was drained from the vessels, and the floating latex layer was drained separately through a 1-mm strainer to catch any coagulated latex. This filtered latex was resuspended with a 1:1 ratio of extraction solution to latex and again adjusted to 0.2% (v/v) CMC for a second rinsing. Six rinses were done on every planting density treatment, resulting in quantifiable volumes of purified latices.

Dry rubber concentration (in mg/mL) was determined from the mean weight of three 0.25-mL aliquots from each latex batch dried for 24 h under a fume hood and by then dividing the rubber weight in mg by the 0.25 mL of latex. Total extracted latex from each treatment was determined by multiplying the dry rubber concentration in mg/mL by the total volume of purified latex.

*Macromolecular characterization of purified latex.* Dried TK dandelion latex (TNRL) films were rolled into dry TK rubber (TNR) rods and dissolved in distilled THF at a concentration of 2 mg TNR/mL. TNR solutions were shaken (IKA MS 3 digital shaker) for 1 week and then filtered through 0.45-µm PTFE syringe filters into SEC vials. A total of 100 µL of each TNR solution was injected into the SEC for analysis. The SEC system consisted of an Agilent 1260 infinity isocratic pump, a Wyatt Eclipse DUALTEC separation system, an Agilent 1260 infinity variable wavelength detector (ultraviolet), a Wyatt OPTILAB T-rEX interferometric differential refractometer, a Wyatt DAWN HELOS-II multiangle static light scattering detector with a built-in dynamic light scattering module, a Wyatt ViscoStar-II viscometer, an Agilent 1260 infinity standard autosampler, and six StyragelVR columns (HR6, HR5, HR4, HR3, HR1, and H0.5) in series. The columns were equilibrated at 35 °C, and THF, continuously distilled from $CaH_2$, was used as the mobile phase at a flow rate of 1 mL/min. Chromatograms were analyzed using ASTRA 7 software (Wyatt Technology, Goleta, CA, USA). Absolute molecular weights were obtained using: $d_n/d_c$ = 0.185 mL/g for the polystyrene standard; $d_n/d_c$ = 0.130 mL/g for *cis*-1,4-polyisoprene. The polystyrene standard was used for quality assurance only. SEC analysis included weight average molecular weight ($M_w$), number averaged molecular weight ($M_n$), polydispersity ($D$), and quantification of the polymer, oligomer, and gel fractions in the TNR dried from the purified TNRL.

*Residual rubber quantification.* The solid root bagasse cake (Section 2.7) from each bed was dried at 50 °C, weighed, and finely ground using an A10 mill (IKA, Wilmington NC). Rubber content was determined by near-IR radiospectrometry (Analytical Spectral Devices Field Spec 3) and an established chemometrics model ($r^2$ = 0.89) based on >300 accelerated solvent extraction reference samples (Cornish et al. 2004; Kopicky and Cornish 2014). This rubber content represents the fraction of rubber that was in solid, coagulated form in the roots during homogenization and so could not be extracted as latex.

*Seed cleaning, quantification, and germination tests.* Seed was separated from the vacuumed mix of TK seed, pappi, dirt, and plant matter (Section 2.6) using a stationary wheat thresher (Precision Machine Co., Lincoln, NE, USA) slightly modified to reduce air flow for the much lighter TK seeds and their associated pappi (Fig. 1). The thresher separated a heavy fraction, primarily seeds and dead flower heads, from a medium weight fraction that was light enough to be pulled through the top of the thresher. The medium fraction outputted near a running flow hood, which served as an additional filter, pulling the lightest components from the medium weight fraction (mostly empty seed cases and pappi) directly out through the hood. The medium weight fraction, which was too heavy to be removed by the flow hood, was recycled back into the thresher at a lower flow speed to better separate light and heavy fractions. Again, the medium fraction was recycled allowing for more fine-tuned adjustments of flow speed until the medium weight collection contained mostly dead flower heads and dirt particles without seed. The heavy fraction, now primarily seeds with a small amount of vacuumed flower heads (Fig. 2A), was passed through sieves with a series of decreasing mesh sizes. The top sieve (Fig. 2B) had 1.68-mm openings and captured all the dead flower heads contained in the heavy fraction. The middle sieve (Fig. 2C) had 0.84-mm openings and caught mostly opened (nonviable) TK seed casings, as well as TK seeds that had failed to separate from their pappi. In this middle sieve, the seeds that did not fall through due to pappi connections were manually rubbed against the sieve mesh to remove their pappi. The final sieve in the stack (Fig. 2D) had 0.50-mm openings and collected the desired,

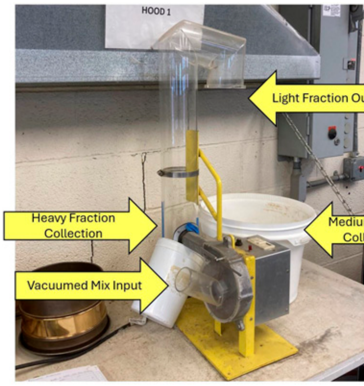


Fig. 1. Modified wheat thresher apparatus used to separate *T. kok-saghyz* seed from their pappi.

clean TK seed, while any broken and empty casings fell through this sieve.

The total weight of clean, collected seed was measured for each bed. A total of 100 seeds from each bed were weighed. The number of seeds collected from each bed was calculated by dividing the total weight of clean seed by the average seed weight.

The viability of this cleaned seed was then evaluated using germination tests. Seeds from each bed were sown into 50-cell flats of Promix FPX biostimulant media and germinated in the same temperature-controlled plastic walled greenhouse as used for the original plant production. Seed germination rates were calculated for 50 seeds from each bed 3 weeks after sowing. The rest of the collected seed was placed in a seed cooling room for 8 weeks at 4 °C to simulate cold stratification. After stratification, 50 seeds from each bed were again sown into 50-cell flats of Promix FPX biostimulant medium and germinated as before. Again, the germination rate out of 50 was recorded to assess whether cold stratification affected germination rates.

*Statistical analysis.* One-way analyses of variance was used to determine the effect of planting density on dependent variables using an alpha level of $P = 0.05$. When significance was found, Fisher's protected least significant difference (LSD) tests were used to generate letter groups and identify values that significantly differed from one another. In tables, wherever possible, the LSD value was provided.

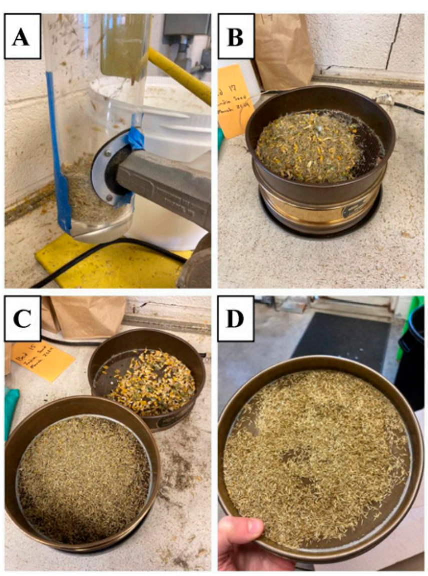


Fig. 2. *T. kok-saghyz* seed collected in the heavy fraction of the seed thresher (**A**), in the heavy fraction at the first level (1.68-mm mesh) of sieving (**B**), and at second level (0.84 mm mesh) of sieving (**C**), and clean seed on the smallest sieve (0.50-mm mesh) (**D**).

## Results

Plants grew well in the greenhouse at all densities. The low-density plants fanned out over the soil, and the more densely planted beds grew more upright (Fig. 3).

*Pest control.* Effective pest control was achieved throughout this planting density study using biological controls in response to pest pressure. *C. rufilabris* (green lacewings) and *A. cucumeris* mites released at time of transplant successfully deterred aphid and thrip populations from establishing (Fig. 4). Two weeks later, *E. formosa* and *E. eremicus* were applied and effectively controlled the developing whitefly presence, while *A. colemani* (parasitic wasps) were released for further aphid prevention. Flowering began in late December and continued until harvest in March. Thrip pests increased with flowering, which is typical given their attraction to pollen, but were controlled with predatory *A. swirskii* mites deployed at the time of flowering (Fig. 4). At the same time, aphid populations began to increase and were immediately addressed using *H. convergens* (convergent lady beetles). This immediate response was required because parthenogenic aphids multiply very quickly and devastate TK greenhouse-grown plants. The *E. formosa* and *E. eremicus* mix was also released at this time and continued to control whiteflies (Fig. 4). Two weeks after this release, *E. formosa* and *C. rufilabris* were released, continuing to control whitefly and aphid, respectively (Fig. 4). In the second half of February, it became clear that the plants were then only facing pressure from thrips and would make it to the early March harvest without further application of beneficial insects (Fig. 4).

*Plant survival rates.* At the lowest planting density of 31 plants/m$^2$, there was 100% survival of root systems, but only 96% of rosettes were recovered, indicating that a few rosettes had died off before harvest, but all root systems were still intact and recoverable (Table 2). There was a significant die off of at least 20% in all other planting densities, and there continued to be fewer rosettes recovered at harvest than root systems. A total of 80% of roots were harvested from both the 62 and 124 plants/m$^2$ densities, and 72% of roots from the 247 plants/m$^2$ density. Thus, despite the decrease in plant survival as planting density increased, there were significantly more roots harvested at the higher densities than the lower ones ($P < 0.001$) (Table 2).

*Root and rosette yields from different planting densities.* The average dry weight of a single rosette, found by dividing total bed dry weight by the number of harvested rosettes, consistently and significantly decreased as planting density increased ($P < 0.001$) (Table 3). However, the average rosette fresh and dry weights per bed were greatest in the lowest and highest planting densities. Water content in the rosettes did not significantly differ among densities, and all were approximately 80%.

The average total dry weight of the roots per bed increased with planting density and was significantly larger at the highest two densities than the lowest two (Table 3). However, weights of single root systems significantly

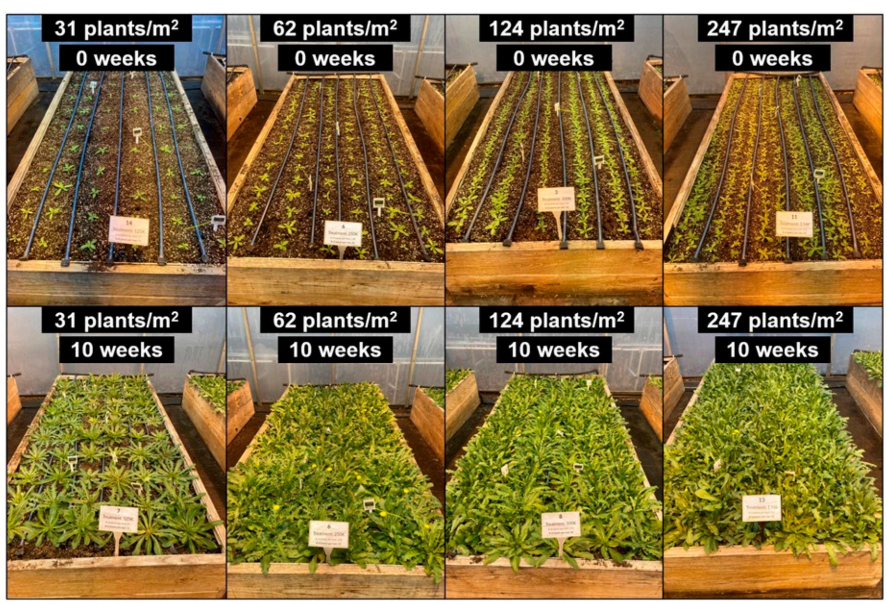


Fig. 3. *T. kok-saghyz* transplants when first planted and after 10 weeks when flowers began opening in all planting densities.

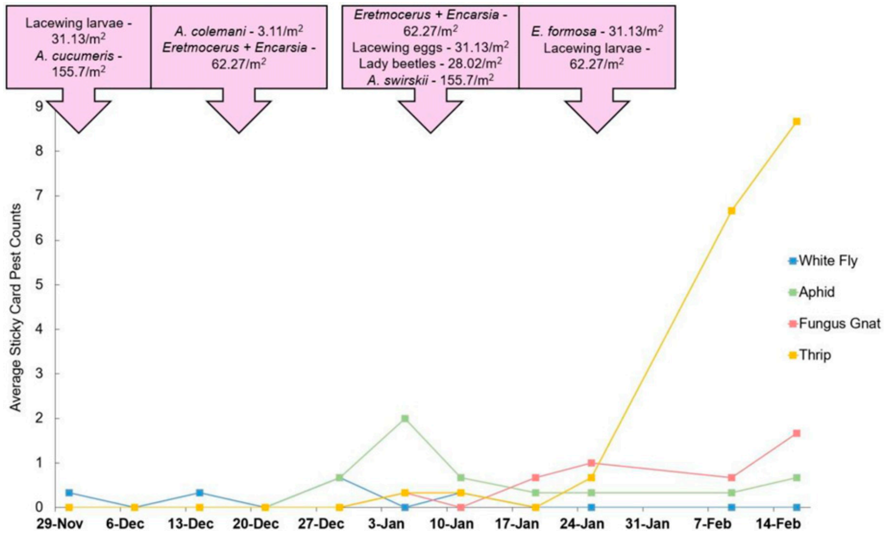


Fig. 4. Timeline of average pest counts on three sticky cards (front, middle, and back of house) throughout greenhouse production and beneficial insects added to the greenhouse to manage the pests.

decreased as planting density increased. Similar to rosettes, root water content was unaffected by planting density at approximately 76%. The root to rosette ratio was lowest at the lowest planting density when rosettes grew the largest. The root to rosette ratio then significantly increased with planting density but declined at the highest (247 plants/m$^2$) density.

*Latex yield.* Homogenate (root slurry filtrate containing latex) volume produced during latex extraction significantly increased as planting density increased (Table 4) caused by the greater amount of root material and greater amount of extraction solution needed. The concentration of latex in the roots was highest at the lowest and highest planting densities (9.92 and 8.53 mg/g dry root, respectively). Extractable latex mirrored this trend and was also highest at the lowest and highest planting densities (4.82 and 3.61 mg/g dry root, respectively). Similarly, more total latex was purified from the lowest and highest planting densities than the middle two. Latex recovery was highest (48%) at 31 plants/m$^2$ density and was slightly lower (approximately 40%) in the other three densities. Additionally, although similar amounts of latex were quantified in the 31, 62, and 124 plants/m$^2$ densities (Table 4, column 5), much more latex was purified from roots at the 31 plants/m$^2$ density (Table 4, column 9).

*Latex quality (SEC characterization).* All extracted TNRL contained high molecular weight TNR as required for product manufacturing (>1 million g/mol) regardless of planting density (Table 5). However, TNR from 31 and 247 plants/m$^2$ had higher gel content than from 62 and 124 plants/m$^2$. All TNRs had similar polydispersities, indicating that distribution of TNR molecular weights was unaffected by planting density.

*Residual dry rubber yields.* Root bagasse weights were significantly higher at the two highest planting densities (124 and 247 plants/m$^2$) than the lowest two planting densities (31 and 62 plants/m$^2$) (Table 6). Thus, because the dry rubber concentration within the bagasse did not change with planting density, the amount of residual dry rubber in the bagasse was significantly higher at the highest two planting densities ($P < 0.01$). The root dry rubber content was highest at 124 plants/m$^2$ at 33 mg/g dry root. Root dry rubber yields were significantly lower than this at the highest and lowest planting densities, opposite to the latex content (Table 4).

*Total (latex + dry) rubber yields.* Fairly similar total rubber concentrations (31 to 38 mg/g dry root) were produced from the latex and rubber extraction processes regardless of planting density (Fig. 5). However, a greater percentage of rubber was extracted in latex form at the lowest and highest planting densities than the middle two densities.

Total rubber per root decreased as planting density increased (Fig. 6A), due to the concomitant reduction in root size. Projected rubber/ha increased as planting density increased up to a maximum of 60.16 kg/ha at a density of 124 plants/m$^2$ but declined at the highest density of 247 plants/m$^2$ (Fig. 6B). Although it was possible to get nearly twice as much latex (15.01 compared with 8.34 kg/ha) at the density of 247 plants/m$^2$, this was at the expense of total rubber, which was highest at 124 plants/m$^2$ (60.16 compared with 57.27 kg/ha).

To visualize the relationship between root mass, rosette mass, and rubber yield per hectare, Supplemental Fig. 1 was created. It is an interactable, three-dimensional representation of the effects of root and rosette yields on rubber yields from all 20 experimental beds (Supplemental Fig. 1). It is clear that an increase in root mass per hectare resulted in increased rubber yields, while rosette mass increases did not result in subsequent rubber yield increases.

The correlation between harvested root mass per hectare and rubber yields seen in Supplemental Fig. 1 was further explored, and it is clear that the two variables are directly correlated ($R^2 = 0.88$) (Fig. 7A) and that rosette yields are not correlated with rubber yields (Fig. 7B).

*Seed yield and viability.* The seeds produced from plants at the lowest planting density (31 plants/m$^2$) were larger than seed from all other plant densities ($P < 0.01$) (Table 7). Regardless, there was no significant difference in the total weight of seed produced per bed or the number of seeds produced per bed. Initial germination tests after seed cleaning showed no significant difference in germination rates, which ranged from 34.8% to 47.6% viability. After seed stratification all germination rates increased 2- to 3-fold and ranged from 79.6% to 92.4%. The planting density of 62 plants/m$^2$ produced the most seed and, at the higher post-stratification germination rate, resulted in noticeably more viable seed (Table 7).

Table 2. Average number of rosettes and root systems harvested from each bed, and their survival rates from initial transplanting.

| Planting density (plants/m$^2$) | Initial no. of plants per bed | Avg. no. of rosettes harvested per bed | Rosette survival rate (%) | Avg. no. of roots harvested per bed | Root survival rate (%) |
|---|---|---|---|---|---|
| 31 | 69 | 66 ± 3 a | 96 a | 69 ± 1 a | 100 a |
| 62 | 138 | 104 ± 7 b | 75 b | 110 ± 5 b | 80 b |
| 124 | 276 | 217 ± 8 c | 79 b | 222 ± 7 c | 81 b |
| 247 | 552 | 397 ± 14 d | 72 b | 400 ± 15 d | 72 b |
| Planting density *P* value | NA | <0.001 | <0.01 | <0.001 | <0.001 |
| LSD value | NA | 27.2 | 11.1 | 26.0 | 8.4 |

Averages are means of five beds ± standard error. *P* values are from a one-way analysis of variance. Fisher's protected least significant difference test was used to generate letter groups. Within a column, numbers with different letters are significantly different at a *P* value of < 0.05 [see least significant difference (LSD)]. The absence of letters under a column means that planting density had no effect on that parameter.

## Discussion

Plant survival rates were nearly 100% at the lowest planting density (31 plants/m$^2$) (Table 2), although this density had the lowest number of harvested plants and the lowest total root dry weight per bed of any treatment (Table 3). The significantly lower root survival rate at the higher densities still resulted in significantly more roots harvested than from the lowest density as planting densities

Table 3. Average fresh and dry weights of rosettes and roots, root:rosette ratios, and water content of individual plants and entire beds at the different planting densities.

| | Bed rosette fwt (kg) | Bed rosette dwt (kg) | Rosette water content (%) | Single rosette dwt (g) | Bed root fwt (kg) | Bed root dwt (kg) | Root water content (%) | Single root system dwt (g) | Root:rosette ratio (dwt) |
|---|---|---|---|---|---|---|---|---|---|
| Planting density | | | | | | | | | |
| 31 plants/m$^2$ | 4.91 ± 0.82 ab | 0.88 ± 0.10 a | 81.55 ± 1.12 | 13.14 ± 1.14 a | 0.85 ± 0.06 d | 0.20 ± 0.01 a | 76.84 ± 0.58 | 2.83 ± 0.16 a | 0.22 ± 0.01 a |
| 62 plants/m$^2$ | 3.42 ± 0.73 bc | 0.63 ± 0.09 b | 80.69 ± 1.17 | 5.96 ± 0.52 b | 1.03 ± 0.04 c | 0.24 ± 0.01 a | 76.24 ± 0.97 | 2.22 ± 0.10 b | 0.39 ± 0.05 b |
| 124 plants/m$^2$ | 3.01 ± 0.10 c | 0.59 ± 0.03 b | 80.29 ± 0.81 | 2.76 ± 0.20 c | 1.21 ± 0.04 b | 0.35 ± 0.05 b | 71.02 ± 3.59 | 1.59 ± 0.21 c | 0.58 ± 0.09 c |
| 247 plants/m$^2$ | 5.16 ± 0.27 a | 0.95 ± 0.07 a | 81.61 ± 0.68 | 2.38 ± 0.09 c | 1.67 ± 0.04 a | 0.39 ± 0.03 b | 76.58 ± 0.93 | 0.98 ± 0.05 d | 0.41 ± 0.02 b |
| Planting density *P* value | <0.05 | <0.01 | 0.72 | <0.001 | <0.001 | <0.001 | 0.1 | <0.001 | <0.01 |
| LSD value | 1.70 | 0.23 | NA | 1.90 | 0.16 | 0.09 | NA | 0.43 | 0.15 |

The values are means of five ± standard error. *P* values are from a one-way analysis of variance. Fisher's protected least significant difference (LSD) test was used to generate letter groups. Within a column, numbers with different letters below them are significantly different at a *P* value of <0.05 (see LSD). The absence of letters in a column means that planting density had no effect on that parameter. dwt = dry weight, fwt = fresh weight.

Table 4. Homogenate latex content, purified latex, percent recovery, and resulting latex yields in mg/g dry root.

| | Homogenate volume produced per bed (mL) | Homogenate latex concn (mg dwt/mL) | Latex concn (mg dwt/g dry root) | Total latex in homogenate per bed (mg dwt) | Total dry latex purified (mg dwt) | Total latex volume purified (mL) | Purified Latex concn (mg/mL) | Total purified latex (mg) | Latex recovery (%) | Recovered latex (mg/g dry root) |
|---|---|---|---|---|---|---|---|---|---|---|
| Planting density | | | | | | | | | | |
| 31 plants/m$^2$ | 1,528 ± 130 c | 1.30 ± 0.10 | 9.92 | 1,944 ± 131 | 9,721 | 27 | 175.07 ± 0.74 a | 4,727 | 48.63 | 4.82 |
| 62 plants/m$^2$ | 1,798 ± 80 c | 0.93 ± 0.28 | 6.67 | 1,629 ± 497 | 8,146 | 24 | 135.73 ± 1.50 b | 3,258 | 40.00 | 2.67 |
| 124 plants/m$^2$ | 2,335 ± 153 b | 0.77 ± 0.22 | 5.27 | 1,862 ± 552 | 9,312 | 23 | 145.60 ± 1.86 b | 3,349 | 35.96 | 1.89 |
| 247 plants/m$^2$ | 3,283 ± 144 a | 1.01 ± 0.22 | 8.53 | 3,351 ± 800 | 16,755 | 49 | 144.93 ± 5.11 b | 7,102 | 42.39 | 3.61 |
| Planting density *P* value | <0.001 | 0.39 | NA | 0.15 | NA | NA | <0.001 | NA | NA | NA |
| LSD value | 390 | NA | NA | NA | NA | NA | 12.0 | NA | NA | NA |

The values for each density are means of five beds ± standard error. All five beds of each density were pooled for latex purification, and latex dry rubber concentrations are means of three aliquots of the purified latex. The *P* values are from a one-way analysis of variance (ANOVA). Fisher's protected least significant difference (LSD) test was used to generate letter groups. Within a column, numbers with different letters below them are significantly different at a *P* value of <0.05 (see LSD). The absence of letters in a column means values were not significantly different or an ANOVA was not possible because the data point is a total of all reps and not an average. dwt = dry weight, NA = not available.

increased (Table 2), as observed previously (Bates et al. 2019). The plant survival rates (with rosettes) in this study's two highest planting densities (124 and 247 plants/m$^2$) were similar (79 and 72%) to those observed previously at the same densities (99 and 62%) (Bates et al. 2019). However, higher die-offs occurred as planting density increased to 4.94 and 9.88 plants/m$^2$, densities at which survival rates were only 48.5 and 32.8%, respectively (Bates et al. 2019). This is possibly because that population was highly diverse and weak plants were aggressively outcompeted under overcrowded conditions. However, the present study found a maximum rubber yield at 124 plants/m$^2$ followed by a decline at a density of 247 plants/m$^2$. Even though this decline was not related to excessive plant die-off, it may reflect the reduced root size observed (Table 3).

Although there were significantly higher bed root dry weights at the higher two planting densities, the dry weight of a single root system significantly decreased with increasing density (Table 3) as the plants became more crowded. In contrast to bed root dry weight, bed rosette dry weight did not increase with planting density (Table 3). Due to having so much space, the average dry weight of a single rosette at the lowest planting density (31 plants/m$^2$) was two to seven times greater than at the higher planting densities, resulting in a total bed rosette dwt similar to the highest planting density. The highest ratio of roots to rosette was 0.58 at the second highest density (124 plants/m$^2$), and notably, this planting density also produced the most rubber per hectare (Fig. 6B). In an earlier study, using smaller plants, 494 plants/m$^2$ produced the most rubber (Bates et al. 2019). Fig. 7 and supplementary Fig. S1 imply that it is larger root yields that should be sought after and selected for to gain more rubber yields, and selecting for rosette biomass to develop more rubber is not advisable (i.e., large rosettes do not equal large amounts of rubber). It appears rosettes of the current TK population are large enough for a wide range of planting densities (Fig. 7B), and maximization of root biomass per hectare, as well as root rubber concentration, should be the breeding foci in the future.

There were several instances in which the lowest and highest planting densities differed from the inner two densities. The rosette dry weights of the beds planted at 31 and 247 plants/m$^2$ were significantly higher than the intermediate densities (Table 3) but again for different reasons. At the lowest planting density, rosettes were able to grow very large, resulting in large total rosette dwts for this treatment. At the highest planting density, rosettes were the smallest on average, but due to the sheer number of plants, there was still a high total rosette dwt.

The significantly larger seed produced by the lowest planting density (Table 7) may have been due to more assimilate from the very large rosettes being used to produce the seed. The flowers themselves were not obviously larger. Despite the vastly different number of plants per bed in each treatment, they did not

Table 5. Macromolecular parameters of *T. kok-saghyz* dandelion natural rubber made by dry *T. kok-saghyz* dandelion latex extracted and purified from roots grown at different planting densities.

| Planting density (plants/m$^2$) | $M_w$ (kg/mol) | $M_n$ (kg/mol) | Polydispersity ($M_w/M_n$) | Oligomer (%) | Soluble polymer (sol) (%) | Insoluble polymers (gel) (%) |
|---|---|---|---|---|---|---|
| 31 | 1515 | 805 | 1.882 | 31.8 | 57.0 | 11.2 |
| 62 | 1600 | 877 | 1.824 | 21.6 | 76.5 | 1.9 |
| 124 | 1255 | 727 | 1.727 | 16.9 | 80.2 | 2.9 |
| 247 | 1744 | 894 | 1.951 | 21.6 | 54.0 | 24.4 |

$M_n$ = number average molecular weight, $M_w$ = weight average molecular weight.

produce significantly different amounts of seed from the same grow area, although the two lower densities may be more productive (Table 7, column 4) due to more flowers per plant. The planting density of 62 plants/m$^2$ produced the most viable seeds per hectare (219.15 million/ha). Also, the buildup of thrips (Fig. 4) seems not to have preferentially affected seed production from one density over another. However, stratification of all seed more than doubled seed germination rates, consistently reaching higher than 80%. This study provides the first estimates of TK seed production rate in a greenhouse environment.

It was unexpected that the amounts of latex present in the homogenate and in purified latex were not correlated with the homogenate volumes produced because total rubber is highly correlated to root size and biomass (Fig. 7A). Thus, planting density clearly affected the synthesis of rubber particles and the persistence of latex present in fresh roots (Table 4). All NR is synthesized in microscopic cytoplasmic rubber particles, and these form a latex emulsion. Solid rubber is formed by the coagulation of rubber particles into solid masses, which in TK form in the living roots especially as they age (Abdul Ghaffar and Cornish 2024), as well as when the roots are dried (King-Smith et al. 2024; Wahler et al. 2009). The highest amount of latex per bed was quantified in beds containing the highest planting density. More latex was purified from roots of 31 plants/m$^2$ density than 62 and 124 plants/m$^2$, despite quantification of similar amounts of latex in the homogenate (Table 4). However, the latex concentration in the homogenate was higher, which may make the latex fraction easier to extract, and the highest recovery rate was observed at this density. Nonetheless, the latex purification creaming method only recovered 35% to 49% of the rubber particles released into the root homogenates, suggesting that some particles were too dense to be purified and so might represent a fraction of small particles with higher protein to rubber and lipid ratios increasing their specific gravity to greater than 1. Heavy particles have previously been found in TK roots (King-Smith et al. 2023).

Purified latex concentration in mg/mL is a function of rubber particle packing density, and the three highest planting densities of this study were in line with previous work (approximately 140 mg/mL) (Table 4, column 8) (King-Smith et al. 2023), whereas the lowest planting density had a significantly higher purified latex concentration (approximately 175 mg/mL). We suggest that the plant-to-plant crowding experienced in the three highest planting densities may have slowed down their maturation rate relative to the lowest planting density. Plants at the lowest density all survived and had much larger root weights per plant than plants at higher densities (Tables 2 and 3). The higher particle packing density in latex purified from roots grown at the lowest planting density (Table 4, column 8) suggests these particles are smaller than those from other planting densities or that the latex contains a larger percentage of smaller particles. We speculate that the roots in the lowest planting density may be physiologically more mature than the others due to lack of crowding. More mature rubber particles may have been translocated into the vacuolar storage compartments, where the number and/or size of the hydration shells surrounding individual particles may have diminished, likely via rubber particle membranes leaching cations into the surrounding aqueous medium, which has been shown to occur naturally over time (King-Smith et al. 2023). The lowest planting density therefore had enough smaller rubber particles (still lighter than water) to affect its latex packing density and may also be the reason for the increased latex recovery at this planting density (Table 4). Unfortunately, particle size was not directly measured in this experiment, and latex samples are no longer available.

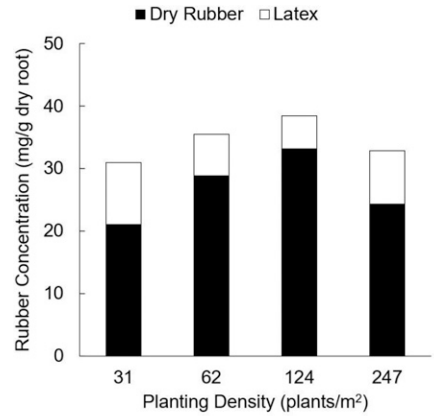


Fig. 5. Rubber (black) and latex (white) yields per gram of dry root at the different planting densities. Total extracted root latex and rubber values were summed for five beds at each density. Bars are the total latex and rubber dry weights divided by the total root dry weight for each planting density.

Latex concentrations in the roots ranged from 5.3 to 9.9 mg/g dry root, on par with previous latex extractions (King-Smith et al. 2022, 2023) but slightly lower, probably because the current roots were a month younger at harvest than the previous ones. The increasing amounts of in vivo latex coagulation as planting density increased over the first three densities could simply reflect the increasing stress caused by crowding and competition (Fig. 5). The increasing amounts of total rubber could also be a stress response. Other stresses such as cold temperatures and wounding have increased root rubber concentration before (Cornish et al. 2013; Dong et al. 2023; Suomela 1950). In contrast, the highest planting density clearly induced a change in the pattern of latex and rubber accumulation that looks more like the pattern of the lowest density (Fig. 5). The crowding competition worsened as evinced by the lower survivability of plants grown at this density (Table 8), and the roots became considerably smaller. At the same time, the TNRL fraction considerably increased (Table 4). It seems possible that competition-induced roots

Table 6. Average root bagasse weights and residual dry rubber contents after latex extraction.

| | Root bagasse wt (g/5 beds) | Bagasse dry rubber concn (mg/g) | Residual dry rubber in bagasse (g/5 beds) | Total dry rubber (mg/5 beds) | Root dry rubber yield (mg/g dry root) |
|---|---|---|---|---|---|
| Planting density | | | | | |
| 31 plants/m$^2$ | 85.12 ± 8.30 b | 49.03 ± 2.74 | 4.2 ± 0.5 c | 20,831 | 21.09 ± 1.40 c |
| 62 plants/m$^2$ | 111.31 ± 3.86 b | 62.69 ± 6.04 | 7.0 ± 0.8 bc | 35,162 | 28.86 ± 3.42 ab |
| 124 plants/m$^2$ | 195.47 ± 36.62 a | 61.47 ± 5.11 | 11.6 ± 1.9 a | 57,861 | 33.18 ± 2.74 a |
| 247 plants/m$^2$ | 169.52 ± 8.65 a | 55.86 ± 3.07 | 9.4 ± 0.6 ab | 47,190 | 24.36 ± 2.18 bc |
| Planting density *P* value | <0.01 | 0.16 | <0.01 | NA | <0.05 |
| LSD value | 58.05 | NA | 3,276 | NA | 7.64 |

The values are means of five ± standard error. The *P* values are from a one-way analysis of variance (ANOVA). Fisher's protected least significant difference (LSD) test was used to generate letter groups. Within a column, numbers with different letters below them are significantly different at a *P* value of <0.05 (see LSD). The absence of letters in a column means the values were not significantly different or an ANOVA was not possible because the data point is a total of all reps and not an average. NA = not available.

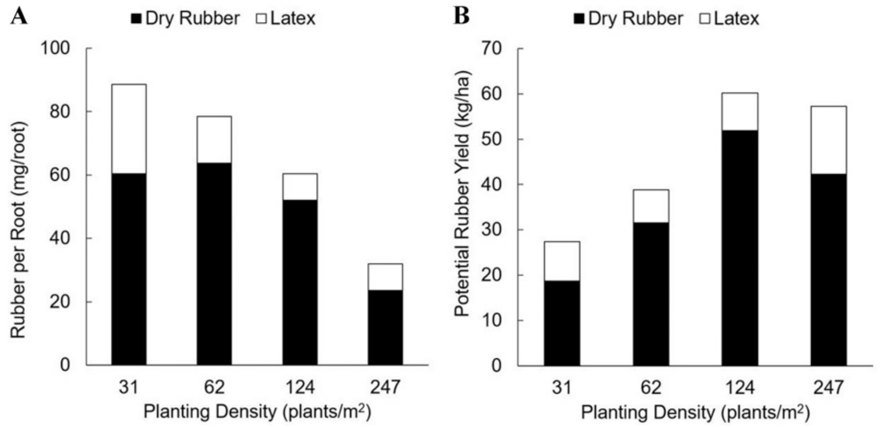


Fig. 6. Rubber (black) and latex (white) per root (**A**) and projected latex and rubber yields (**B**) at the different planting densities. Rubber per root values (**A**) are total latex and rubber yields for each planting density divided by the sum of harvested roots. Rubber per hectare values (**B**) represent total latex and rubber yields from each density divided by the total grow area for each treatment, scaled to kilograms per hectare.

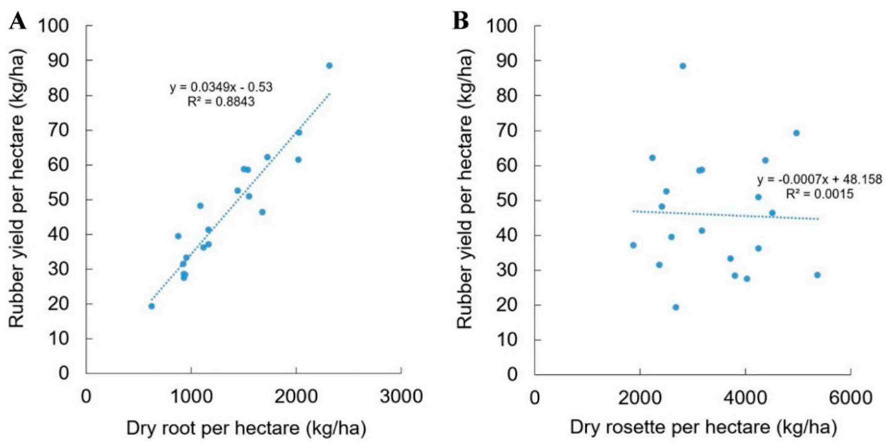


Fig. 7. Relationship between root yields (**A**), rosette yields (**B**), and potential rubber yields per hectare for 20 experimental beds.

were not only smaller but were also more juvenile in development and not yet capable of inducing as much TNRL coagulation as more mature roots (Abdul Ghaffar and Cornish 2024). In the earlier study, latex rubber fraction decreased as planting density increased, and at their maximum density, latex fraction increased again (Bates et al. 2019).

In parallel with the amount of TNRL produced (and purified), the TNR made from these latices contained less soluble (sol) rubber (able to dissolve in strong organic solvents) and much more insoluble rubber (gel) than that from intermediate planting densities (Table 5). Because gel forms within the rubber particles by a poorly understood spontaneous polymer aggregation and cross-linking process, it may be that the inhibited coagulation process inferred left these particles in an active state for significantly longer than the rapidly coagulated particles. This longer time could have led to higher gel formation. There is no evidence that gel content changes in rubber after it is coagulated and dried.

The TNR (from TNRL) was all of high molecular weight (Table 5) and in line with previous macromolecular characterization of dried TNRL films (King-Smith et al. 2023). Although the previous macromolecular characterization of TNR found approximately 30% gel (King-Smith et al. 2023), the gel percentage in the present study was lower (2% to 24%). Regardless, at all planting densities tested in this work, extracted and purified TNRL was of sufficient quality to make rubber products (King-Smith et al. 2024).

Potential rubber yield as a function of area indicated that 124 plants/m$^2$ would produce about 60 kg TNR/ha (Fig. 6). This is considerably below one earlier report (Bates et al. 2019) but higher than others (Arias et al. 2016; Eggert et al. 2018; Kreuzberger et al. 2016; Van Beilen and Poirier 2007). These reports were carefully studied to isolate potential causes of the reported differences, and key parameters were tabulated (Table 8).

The "reported total rubber yields" row (Table 8) lists the reported yields from all studies. The values used for Bates et al. (2019) are modified because we think that they overestimated their projected rubber yield by a factor of 4; the error occurs in Fig. 9 of their paper, where they refer to rubber per total plot as the rubber yield per quarter plot. Furthermore, they based their projections on the larger roots ($\geq$7 g fwt) in their highly diverse population. Thus, the "calculated rubber yields" row standardized all studies by including roots of all sizes. This calculation required harvest density (planting density $\times$ survival rate), average dry root weight of a single root, and root rubber concentration (Eqs. [1] and [2]).

$$\frac{\#\,\text{of plants}}{\text{ha}} \times \frac{\#\,\text{gram}}{\text{single dry root}} \times \frac{1\,\text{kg}}{1000\,\text{g}} = \frac{\text{kg dry roots}}{\text{ha}} \qquad [1]$$

$$\frac{\text{kg dry roots}}{\text{ha}} \times \frac{1000\,\text{g dry roots}}{1\,\text{kg dry roots}} \times \frac{\#\,\text{mg rubber}}{\text{g dry roots}} \times \frac{1\,\text{g rubber}}{1000\,\text{mg rubber}} \times \frac{1\,\text{kg rubber}}{1000\,\text{g rubber}} = \frac{\text{kg rubber}}{\text{ha}} \qquad [2]$$

Table 7. Seed production results for the different planting density treatments.

| | Seed wt (mg/seed) | Produced seed per bed (g) | No. of seeds per bed | Prestratification seed gemination (%) | Post stratification seed germination (%) | No. of germinable seeds per bed | No. of germinable seeds per hectare (millions/ha) |
|---|---|---|---|---|---|---|---|
| Planting density | | | | | | | |
| 31 plants/m$^2$ | 0.68 ± 0.04 a | 30.05 ± 3.6 | 46,366 ± 5,591 | 42.8 ± 10.1 | 79.6 ± 3.2 b | 37.902 ± 5017 | 169.66 ± 25.1 |
| 62 plants/m$^2$ | 0.58 ± 0.05 ab | 28.51 ± 3.0 | 52,876 ± 7,048 | 34.8 ± 1.4 | 92.4 ± 2.3 a | 48,959 ± 7,125 | 219.15 ± 35.7 |
| 124 plants/m$^2$ | 0.48 ± 0.02 b | 19.88 ± 2.5 | 41,629 ± 5,188 | 39.2 ± 5.6 | 87.6 ± 1.5 ab | 36,197 ± 4,116 | 162.03 ± 18.4 |
| 247 plants/m$^2$ | 0.56 ± 0.02 b | 23.75 ± 2.6 | 43,139 ± 5,705 | 47.6 ± 7.5 | 82.0 ± 3.5 b | 35,539 ± 5,175 | 159.08 ± 23.2 |
| Planting density *P* value | <0.01 | 0.09 | 0.57 | 0.615 | <0.05 | 0.36 | 0.36 |
| LSD value | 0.0001 | NA | NA | NA | 8.3 | NA | NA |

The values are means of five ± standard error. The *P* values are from a one-way analysis of variance. Fisher's protected least significant difference (LSD) test was used to generate letter groups. Within a column, numbers with different letters below them are significantly different at a *P* value of < 0.05 (see LSD). The absence of letters in a column means values were not significantly different.

It is clear that the corrected values from Bates et al. (2019) still show substantially higher rubber yield (162 kg/ha) than has been reported elsewhere (Table 8). The only other yields of this magnitude (150 to 500 $kg \cdot ha^{-1} \cdot yr^{-1}$) were reported in a review article (Van Beilen and Poirier 2007) that cited several works in which the highest reported yield we could find was 54 kg/ha (Whaley and Bowen 1947). Thus, the 150 to 500 kg/ha of TK rubber claimed by Van Beilen and Poirier (2007) could not be verified.

Multiple parameters affect projected yields/ha. High planting densities are clearly needed (Table 8), and plant age at harvest is key: older plants have more rubber in their roots than younger ones. Roots from the study of Bates et al. (2019) were 2 months older than those in the current investigation, supporting the large increase in root dwt reported (1.5 to 2 times bigger), as well as higher average rubber concentration in the roots (from approximately 3% to approximately 5%), which in combination resulted in twice the amount of rubber in the roots (Table 8). The extra 2 months of growth between 4 and 6 months are indeed significant (Arias et al. 2016), when, even at their very low planting density, total rubber yields doubled with just 1 extra month of growth. Yields were also much higher in 6-month-old roots at (0.22 million plants/ha) (Eggert et al. 2018) (Table 8) than in younger ones. When roots from Kreuzberger et al. (2016) (5 months old, 0.08 million plants/ha) were compared with roots a month older from Eggert et al. (2018) (6 months, 0.09 million plants/ha), rubber yields tripled in this extra month of growth (Table 8). OSU researchers also observed that root size and rubber concentration both doubled in November when field-grown (Cardina J, Robinson B, Cornish K, unpublished results).

Because our roots were from an eighth generation population (India) of TK bred and selected for large root size in the field, it is likely that, at 6 months old, they would have been considerably larger than the 6-month-old roots produced at similar planting densities by Bates et al. (2019). This would have led to projected yields higher than we calculated from their data. The effects of this breeding selection can be seen when comparing India TK at 0.62 million plants/ha with the Kreuzberger et al. (2016) planting density of 0.5 million plants/ha (Table 8). When our roots were 4 months old and theirs were 5, our roots were larger with greater rubber yield at the same harvest density. Rubber yields of the 4-month-old India roots were also greater (39 kg/ha) than 12-month-old roots (36 kg/ha) grown at the same density (Table 8), although it must be noted that they used a mechanical harvester at a depth of 0.1 to 0.15 m and may have left some roots behind during harvest (Kreuzberger et al. 2016).

It is clear that planting densities tested in the three previous reports (Arias et al. 2016; Eggert et al. 2018; Kreuzberger et al. 2016) are much too low for commercial rubber production. When comparing 4-month-old roots from our study to Arias roots, rubber yields

Table 8. Comparison of recent planting density trials for *T. kok-saghyz* and its projected rubber yields in kg/ha.

| Study | Arias et al. (2016) | | Eggert et al. (2018) | | Kreuzberger et al. (2016) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Study year | 2009 | | 2012–13 | | 2012–14 | | | | | |
| Population | USDA Kazakhstan collection of 2008 | | USDA Kazakhstan collection of 2008 | | USDA Kazakhstan collection of 2008 | | | | | |
| Production method | Field | | Field | | Field | | | | | |
| Soil type | Loamy soil | | Chernozem with loamy texture | | Chernozem with loamy texture | | | | | |
| Harvest method | Pulled whole roots | | Cutting the roots in a depth of 0.15–0.25 m | | Cutting the roots in a depth of 0.1–0.15 m | | | | | |
| Plant age (mo.) | 4 | 5 | 6 | | 5 | | 12 | | 17 | |
| Planting density (million plants per ha) | 0.24 | 0.24 | 0.09 | 0.22 | 0.08 | 0.5 | 0.08 | 0.5 | 0.08 | 0.5 |
| Plant survival (%) | | | 67 | 55 | 100 | 100 | 100 | 84 | 100 | 44 |
| Harvest density (million plants per ha) | | | 0.06 | 0.12 | 0.08 | 0.5 | 0.08 | 0.42 | 0.08 | 0.22 |
| Single root dry wt (g) | 3.48 | 5.85 | 6.8 | 5.2 | 4.5 | 1.45 | 4.2 | 1.4 | 6.8 | 3.2 |
| Dry root weight per hectare (kg/ha) | 835 | 1404 | 408 | 624 | 360 | 725 | 336 | 588 | 544 | 704 |
| Rubber concn (mg/g dry root) | 26 | 33 | 66 | 54 | 36 | 41 | 77 | 61 | 44 | 56 |
| Rubber per root (mg/root) | 90 | 195 | 450 | 280 | 160 | 60 | 325 | 85 | 300 | 180 |
| Reported total rubber yields (kg/ha) | 23 | 46 | 32 | 37 | 10 | 28 | 23 | 36 | 22 | 42 |
| Calculated rubber yields (kg/ha) | 22 | 47 | 27 | 34 | 13 | 30 | 26 | 36 | 24 | 39 |

| Study | Bates et al. (2019) | | | | | | | | King-Smith et al. (2024) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study year | 2013 | | | | | | | | 2023–24 | | | |
| Population | Second generation progeny from USDA Kazakhstan collection of 2008, selected and bred for large root size in the field at Ohio State University | | | | | | | | Ninth generation progeny from USDA Kazakhstan collection of 2008, selected and bred for large root size at Ohio State University | | | |
| Production method | Outdoor raised beds | | | | | | | | Greenhouse raised beds | | | |
| Soil type | Soilless media: Promix BX | | | | | | | | Soilless media: Promix BX mycorrhizae | | | |
| Harvest method | Pulled whole roots | | | | | | | | Pulled whole roots | | | |
| Plant age (mo.) | 6 | | | | 12 | | | | 4 | | | |
| Planting density (million plants per ha) | 1.24 | 2.47 | 4.94 | 9.88 | 1.24 | 2.47 | 4.94 | 9.88 | 0.31 | 0.62 | 1.23 | 2.47 |
| Plant survival (%) | 99 | 62 | 49 | 33 | 49 | 29 | 28 | 16 | 100 | 80 | 81 | 72 |
| Harvest density (million plants per ha) | 1.22 | 1.52 | 2.40 | 3.24 | 0.60 | 0.71 | 1.38 | 1.55 | 0.31 | 0.50 | 1.00 | 1.78 |
| Single root dry wt (g) | 2.7 | 2.04 | 1.5 | 1.36 | 2.2 | 1.96 | 1.8 | 1.34 | 2.83 | 2.22 | 1.59 | 0.98 |
| Dry root weight per hectare (kg/ha) | 3301 | 3104 | 3594 | 4407 | 1326 | 1385 | 2490 | 2079 | 878 | 1094 | 1583 | 1759 |
| Rubber concn (mg/g dry root) | 37 | 35 | 45 | 33 | 43 | 51 | 43 | 51 | 31 | 36 | 38 | 33 |
| Rubber per root (mg/root) | 100 | 71 | 68 | 45 | 95 | 100 | 77 | 68 | 89 | 78 | 60 | 32 |
| Reported total rubber yields (kg/ha) | 188[i] | 214[i] | 438[i] | 339[i] | 134[i] | 179[i] | 348[i] | 464[i] | 27 | 39 | 60 | 57 |
| Calculated rubber yields (kg/ha) | 122 | 109 | 162 | 145 | 57 | 71 | 107 | 106 | 27 | 40 | 60 | 58 |

[i] The values for roots are >7 g fresh weight.

Studies include Arias et al. (2016), Bates et al. (2019), Eggert et al. (2018), and Kreuzberger et al. (2016), as well as the work of this study (King-Smith et al. 2024). USDA = US Department of Agriculture.

increased with planting density, at least up to a density of 1.23 million plants per hectare, five to six times greater than the planting density they attempted (Arias et al. 2016). Kreuzberger et al. (2016) noted that limitations in planting density based on available seed sourced from the USDA were encountered, and similar issues may have been encountered by the other groups. More seed was available to the OSU studies, which has produced millions of seeds from populations selected from the original USDA seed collection. Although the highest rubber yields (at 6 months old) were reported at a density of 4.94 million plants/ha from the early heterogeneous germplasm (Bates et al. 2019), we believe the ideal density for the much larger [10 times bigger (Wheeler B, Fresnedo-Ramirez J, Cornish K, unpublished observation)] India population now lies closer to 1.23 to 2.47 million plants/ha. Also, planting at densities above 2.47 million plants/ha would result in serious overcrowding die off (Bates et al. 2019), subsequent spreading of plant molds, and potentially create a breeding ground for pests, such as green aphids, that would be nearly impossible to control under such a thick crop canopy.

TK root and rubber yields currently require as long a growing season as possible in soil/soilless medium before harvest. Bates et al. (2019) analyzed plants that had grown for 2 months in trays before being transplanted to raised beds for an additional 6 months and so were effectively 8 months old when harvested in late autumn. Overwintering to increase plant age has been considered but was found to reduce TK populations by at least 50%, so is not currently a promising approach for areas with cold and snowy winters (Bates et al. 2019; Kreuzberger et al. 2016) (Table 8).

## Conclusions

High root and rubber yields in annual TK crops are supported by high planting density, long growing seasons, and late harvest. Optimal planting density is directly related to plant size and so must be determined for specific populations, with a high root:rosette ratio being most likely to maximize rubber yields. Stratification doubled seed germination rates and is recommended for all TK seed production scenarios after seed cleaning. Planting density does not affect rubber molecular weight but does affect root biomass, rubber and latex yield, and gel content, as well as seed number and quality. The effective biocontrol of insect pests prevalent in greenhouse TK production provides guidance to potential organic growers and was achieved by close monitoring of insect pests and with rapid biological responses.

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