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ARTICLE

Eight cycles of half-sib family recurrent selection to improve rubber yield in Russian dandelion

David J. Wolyn and Gregory Innes

Abstract: Russian dandelion (*Taraxacum kok-saghyz*) produces natural rubber which can complement the world's supply of this strategic commodity, derived mainly from the Para rubber tree (*Hevea brasiliensis*). Four cycles of half-sib family recurrent selection conducted previously in Russian dandelion improved rubber yield nearly 50%. The objectives of this research were to continue selection for four additional cycles and evaluate progress from eight generations of breeding, and assess the potential for future gains. Rubber yield increased from 0.205 to 0.378 g/plant from Cycle 0 (C0) to C8, representing an 84% improvement, or 10% per cycle. Increases from C0 to C4 were similar to those from C4 to C8. Root dry weight did not increase from selection and improved yield only resulted from enhancing rubber percentage which changed from 4.35% to 7.62%. Selection response has not plateaued, and phenotypic variation has not decreased, indicating continued gains can occur with additional breeding.

Key words: Taraxacum kok-saghyz, rubber percentage, rubber yield, root dry weight, breeding.

Résumé : Le pissenlit russe (*Taraxacum kok-saghyz*) sécrète un caoutchouc naturel dont on pourrait se servir pour augmenter la production mondiale de ce matériau stratégique, principalement tiré de l'hévéa (*Hevea brasiliensis*). Quatre cycles de sélection récurrente au sein d'une descendance uniparentale antérieurement réalisés sur le pissenlit russe avaient permis d'en augmenter la production annuelle de caoutchouc de près de 50 %. Les auteurs ont voulu poursuivre le travail quatre cycles de plus pour évaluer les progrès obtenus après huit générations de croisements et déterminer si d'autres gains seraient réalisables à l'avenir. Entre les cycles zéro et huit, le rendement en caoutchouc est passé de 0,205 à 0,378 g par plante, ce qui représente une hausse de 84 %, soit de 10 % par cycle. La hausse observée entre les cycles C0 et C4 était semblable à celle relevée entre les cycles C4 et C8. L'hybridation n'augmente pas le poids sec des racines et on ne doit le meilleur rendement qu'à la plus forte proportion de caoutchouc dans la sève, car elle passe de 4,35 à 7,62 %. La réaction à la sélection n'a pas atteint de plateau et la variation du phénotype n'a pas faibli, signe que de nouveaux croisements pourraient entraîner d'autres gains. [Traduit par la Rédaction]

Mots-clés : Taraxacum kok-saghyz, proportion de caoutchouc, rendement en caoutchouc, poids sec des racines, hybridation.

Introduction

Natural rubber is a strategic commodity obtained primarily from the Para rubber tree (*Hevea brasiliensis*) cultivated in southeast Asia (van Beilen and Poirier 2007). The world supply from this one species is vulnerable to climate change, land conversion to other crops, concentrated production in one geographic region, and disease due to low genetic diversity (van Beilen and Poirier 2007). With inferior properties, synthetic rubber cannot replace the natural form in many manufacturing applications, which includes over 50 000 products ranging from tires to medical equipment (Cornish 2017). To alleviate risk associated with relying solely on the rubber tree for natural rubber, alternative crops have been identified.

Russian dandelion (*Taraxacum kok-saghyz*) produces natural rubber with properties similar to that derived from the rubber tree (Whaley and Bowen 1947). Native to Kazakhstan, the species is adapted to temperate climates, including those in Europe and North America (Krotkov 1945; Cornish 2017). Russian dandelion is diploid ($2n = 16$), perennial and cross-pollinating. (Warmke, 1943). Outcrossing is enforced through self-incompatibility, likely a sporophytic mechanism which common to

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the Asteraceae (Allen et al. 2011; Wollenweber et al. 2021). Consequently, a breeding method such as recurrent selection is highly applicable to the crop for population improvement.

Extensive research on this species was conducted during World War II as rubber supplies from southeast Asia diminished (Whaley and Bowen 1947), however, breeding and agronomic studies ceased after the war, when export of natural rubber from the region resumed, and improved germplasm was lost. Wild populations from that era had 4.4% rubber and breeding in Russia suggested 15% was achievable (Whaley and Bowen 1947; Koroleva 1940, as cited in Whaley and Bowen 1947), however, the high level may have represented an improved line or family rather than a breeding population (Hodgson-Kratky et al. 2017).

Recent supply constraints, increased prices, and predictions of future shortages for natural rubber heightened interest in alternatives to the rubber tree (Cornish 2017) and prompted collection of several Russian dandelion accessions from Kazahhstan by the USDA–ARS National Plant Germplasm System in 2008 (Hellier 2011). Using this material, half-sib family recurrent selection for rubber yield was initiated in two populations. One adapted to loam and another adapted to sandy soil, to broaden the area where the crop could be grown, including the sandplains of southern Ontario, Canada (Hodgson-Kratky et al. 2017). Although no response was observed on sandy soil, rubber yield increased nearly 50% after four selection cycles, Cycle 0 (C0) to C4, for the population grown on loam. This response was only achieved by improving rubber percentage from 4.17% to 6.4%; root dry weight did not change and had zero heritability.

Recurrent selection has potential to improve populations incrementally over many cycles provided genetic variation is not exhausted. Continuous gains were observed over 100 cycles in maize where oil percentage increased five-fold (Dudley 2007). Successful long-term response requires not only sufficient genetic variation in the base population, but also considerations of population size and selection intensity. High selection intensities and low populations sizes can limit the amount of genetic variation carried forward for long-term gain. Strong selection pressure in large populations, however, provides an opportunity to maximize gain while minimizing loss of genetic diversity.

The objective of this research was to assess the response of continued recurrent selection in the Russian dandelion population adapted to loam soils developed by Hodgson-Kratky et al. (2017). Selection was conducted for four additional cycles to C8; significant gains were achieved, and continued response is predicted in the future.

Materials and Methods

Breeding strategy – C0 to C8 populations

A half-sib family recurrent selection experiment was initiated and conducted for four cycles (C0 to C4) as described previously, where a base population was developed, and selection occurred to develop populations adapted to sand and loam soils separately (Hodgson-Kratky et al. 2017). For each population and cycle, approximately 100 families were evaluated in two blocks on their respective soil types with a randomized complete block design. Each plot contained 24 plants and two to six C0 control plots were grown in each block. The 10 families with the greatest rubber yield (~10%) were selected and 10 plants from each family chosen randomly. Mating 100 plants generated 100 half-sib families for subsequent selection in each of the sand- and loam-adapted populations.

The breeding process was modified beginning with the C4 populations when selection continued only for that developed previously on loam soil. Populations and C0 controls were grown in two blocks at each of two sites in different areas of the Simcoe Research Station, Simcoe, ON, Canada (lat. 42° 51′ N, long. 80° 16′ W, elevation 240.5 m). Four C0 control plots were grown in each block. From the C4 population of 90 families, 20 were selected. Ten random plants from each family were chosen and 200 individuals collectively intermated to generate 200 half-sib families constituting the C5 population. For the C5 and subsequent populations, 20 families were selected, and 200 families generated. Due to plant loss during pollination less than 200 families were planted: C5 = 139, C6 = 121, C7 = 193. Consequently, selection intensities were 14%, 17%, and 10%, respectively. Breeding concluded with the development of the C8 population. Only bulked seed across C8 families was assessed as described below in the Genetic Gain Experiment, and individual families were not planted.

For the C4 population of half-sib families and subsequent generations, plant culture in the greenhouse and field, harvesting, root processing and pollination were similar to the methods of Hodgson-Kratky et al. (2017) with the following exceptions. Venture® L was sprayed to control grasses on 6 Aug. 2018 for the C6 population (1.5 L·ha^{-1}) and 26 Jun. 2019 for the C7 population (1.0 L·ha^{-1}). After harvest plants were stored for short periods at 4 °C during processing. From each plot 14 plants were allocated for rubber determination and 10 were stored for subsequent crossing. In addition, the C0 control population was regenerated as needed by randomly mating 200 plants to maintain allelic diversity.

For plants used in rubber analysis, leaves were removed completely and roots dried at 45 °C for 3 to 5 d, followed by grinding in a Waring Blender (Waring 7010S: Conai Corp., East Windsor, NJ). Powder was used to estimate rubber percentage using near-infrared spectroscopy (NIR) (Foss Analytics NIRS™ DS2500 Analyser, Foss Analytics, Nils Foss Allé 1, DK-3400 Hilleroed, Denmark) as described below. Grinding occurred immediately after removal from the dryer and NIR determinations made within 2 to 4 h of grinding to prevent absorption of moisture and clumping of powder.

Roots of plants for crossing were cleaned with compressed air and leaves cut within 3 to 5 cm of the crown. Plants were placed in paper bags with softwood shavings and stored at 4 °C until selections were planted in late-December.

Genetic gain experiment

Seed was bulked among families for each of the C0, C4 and C8 populations and planted in the greenhouse from 20 Apr. to 12 May 2020, in four blocks over time. Prior to planting, seed was primed with KH_2PO_4 (18 g·L^{-1}) according to Duray and Davies (1994). Plantlets were then established as described previously (Hodgson-Kratky et al. 2017). Transplants were grown at two sites on each of the Woodstock (W1 and W2) (lat. 43° 8′ N, long. 80° 47′ W, elevation 305 m) and Simcoe (S1 and S2) Research Stations, Ontario, Canada, planted on 8 and 9 June 2020, respectively. Soil types at Simcoe were loamy sand and fine sandy loam on sites S1 and S2, respectively, and Woodstock sites were both loam.

A randomized complete block design with four blocks and the three populations as treatments was used at each site. Plots contained 100 plants spaced 5 cm with 1 m between rows. Due to low germination of the C4 population, plot size was reduced to 50 plants, and four replicate plots could not be established at all sites. Locations were fertilized according to recommendations for carrot [*Daucus carota* ssp. *sativus* (Ontario Ministry of Agriculture, Food and Rural Affairs 2010)]. Plots at sites S2, W1 and W2 were irrigated at planting, and during the growing season when necessary to minimize drought stress. Those on the loamy sand at site S1 were watered at planting and 2.5 cm was applied weekly using trickle tape if rainfall was insufficient; in addition, they received 5.0 cm for each of two weeks during extreme heat in July. Plants were harvested mechanically with a potato digger (Niplo D-65A; Matsuyama Co., Ueda, Japan) on 3 and 4 Nov. 2020 at the Simcoe and Woodstock sites, respectively.

Rubber analysis

Rubber percentage was determined with near-infrared (NIR) spectroscopy using a predictive model developed from rubber concentrations of 350 samples that were estimated gravimetrically by solvent extraction (Hodgson-Kratky et al. 2017). NIR spectra were then determined, and a predictive model developed with WinISI software. A scores file was created using the collected spectra data and plotted in 3-D; outliers were identified, rescanned, and removed as necessary. A global equation was developed with full spectrum which had the smallest standard error of calibration (SEC) value, an R^2 (RSQ) value close to 1, the smallest standard error of cross validation (SECV) value, and an estimate of *R*-square (1-VR) value close to 1. Rubber yield per plant was estimated as [average root dry weight per plant × (rubber percentage/100)]. Accuracy was confirmed using samples with known rubber concentrations not used in the predictive model development.

Statistical analysis

Rubber percentage, root dry weight and rubber yield were assessed by Analysis of Variance (ANOVA) with PROC GLIMMIX in SAS (version 9.4; SAS Institute, Cary, NC). Population was a fixed effect and both site and blocks nested within site were random effects. The Shapiro-Wilk statistic, Levene's test and residual plots were used to test the assumptions of the ANOVA. A likelihood ratio test was used to determine if the random interaction between site and population differed from zero. Least square means were separated using Tukey's honestly significant difference ($P \leq 0.05$). Pearson correlation coefficients between rubber percentage and root dry weight were estimated for each population across all sites and blocks using PROC CORR.

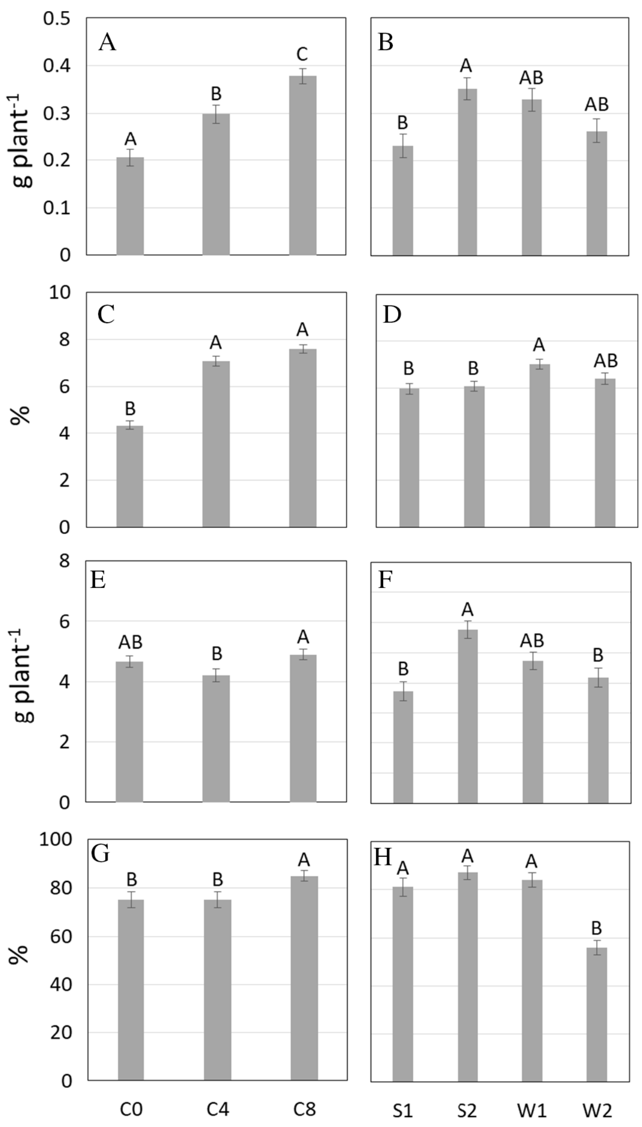
Results

For the 2020 field experiment comparing bulked seed of C0, C4 and C8 populations, the analysis of variance indicated the effects of population and site were significant for rubber percentage and yield, percent survival and plant dry weight. No population × site interactions were observed; therefore, data were pooled.

Rubber yield increased 84%, from C0 (0.205 g·plant^{-1}) to C8 (0.378 g·plant^{-1}), with similar incremental gains from C0 to C4 and C4 to C8 (Fig. 1A). Rubber percentage increased 75% over eight selection cycles, relative to the C0, however, most gain occurred from C0 to C4 (Fig. 1C). Root dry weight did not change from C0 to C8 and values for C4 were lower than expected (Fig. 1E). Survival for the populations ranged 75% to 85% (Fig. 1G). Gains per cycle from regression analysis, expressed as a percentage of the C0 were 9.3%, 10.5% and 0.6 % for rubber yield, rubber percentage and root dry weight, respectively. All traits varied across locations with the greatest disparities for root dry weight and rubber yield (Figs. 1B, 1D, 1F, and 1H).

Analysis of half-sib family populations in their respective years of growth during the selection experiment indicated similar overall gains compared with the 2020 field experiment (Fig. 2). C7 rubber yield and rubber percentage were 70% and 74% greater than the C0, respectively (Figs. 2A and 2B). Root dry weight was variable over cycles, fluctuating above and below the 100% value, relative to the C0 control (Fig. 2C). Slopes estimated from regression analysis, representing percent gain per cycle relative to C0 were 12.4, 11.1 and 0.03 for rubber yield, rubber percentage and root dry weight,

Fig. 1. Response to selection in Russian dandelion for rubber yield (A, B), rubber percentage (C, D), root dry weight (E, F), and survival (G, H) for populations Cycle 0 (C0), C4, and C8 (A, C, E, G), and locations (B, C, F, H). Locations: S1 and S2 — sites 1 and 2 at the Simcoe Research Station, Ontario, Canada; W1 and W2 — sites 1 and 2 at the Woodstock Research Station, Ontario, Canada. Means ± SE ($n = 15$ C0, $n = 12$ C4, $n = 16$ C8, $n = 10$ S1, $n = 12$ S2, $n = 11$ W1, $n = 10$ W2) are presented pooled over populations and sites. Letters indicate significance according to Tukey's honestly significant difference ($P \leq 0.05$).



respectively, and were similar to estimates from the 2020 experiment.

For selection occurring in the C4 to C7 generations, from 2016 to 2019, maximum variation among years for the C0 control was 89%, 48%, and 58% for rubber yield, rubber percentage and root dry weight, respectively (Table 1). Means of selected families over the four breeding cycles averaged 46%, 19%, and 27% greater than their respective population means for the three traits, respectively. Selection differentials expressed relative to population standard deviations and coefficients of variation were variable for the three traits, suggesting selection was not decreasing these parameters and variation remains for future breeding advances.

Discussion

Eight cycles of half-sib family recurrent selection increased rubber yield approximately 10% per cycle in Russian dandelion. The trait is the product of rubber percentage and root dry weight and only the former responded to breeding. Variation did not appear to narrow with selection and gains should continue with further breeding. Rubber yield varied across locations and was likely dependent on soil type and water status.

For the 2020 experiment, rubber yield increased at similar levels from C0 to C4 and C4 to C8, whereas a diminished response was observed for rubber percentage from C4 to C8 (Fig. 1A). Although selection intensity from C4 to C8 averaged 16% compared with 10% for prior cycles, this difference cannot account for the variable gains. Root dry weight was lowest for the C4 population. Some C4 seed used in the experiment had low germination and likely resulted in reduced seedling vigor and harvested root dry weight compared with other populations. Since rubber percentage and root dry weight were correlated negatively in the C4 ($r = -0.5$) (data not shown), the low C4 root dry weight may have inflated the rubber percentage, exaggerating the gain from C0 to C4 and underestimating that from C4 to C8. This compensation between root dry weight and rubber percentage, ultimately resulted in C4 rubber yield that was intermediate to C0 and C8. Analysis of individual populations in separate years indicated similar linear increases for rubber percentage both before and after the C4 generation (Fig. 2), further suggesting the C4 rubber percentage data in the 2020 field experiment was an anomaly.

Both C0 and C4 populations were evaluated previously (Hodgson-Kratky et al. 2017). In that experiment the C4 was improved 45% and 53% compared with the C0 for rubber yield and rubber percentage, respectively. In the 2020 field experiment rubber yield was improved 45%, similar to that of the past assessment. Rubber percentage reported here increased 63% between C0 and C4, or 10% greater than that described earlier, further indicating the 2020 C4 rubber percentage may be inflated from low root dry weight.

Differences were detected among location means for all traits. Rubber percentage was least affected while rubber yield and root dry weight were most altered, as observed previously (Hodgson-Kratky et al. 2017). Since rubber yield is the product of rubber percentage and root dry weight, variation in yield can be explained mostly by location effects on root mass. Root dry weight was lowest at the S1 loamy sand location in Simcoe that

Fig. 2. Selection response over seven generations for Russian dandelion breeding populations planted in different years for (A) rubber yield, (B) rubber percentage and (C) root dry weight. Response was calculated as (Cn/C0 × 100) in each year, where C0 represents the base breeding population (control) and Cn indicates improved populations after *n* breeding cycles, planted in subsequent years at the Simcoe Research Station, Ontario, Canada. Mean ± SE are presented ($n = 2$ for C0 to C3 and $n = 4$ for C4 to C7). Populations were planted from 2012 to 2019.

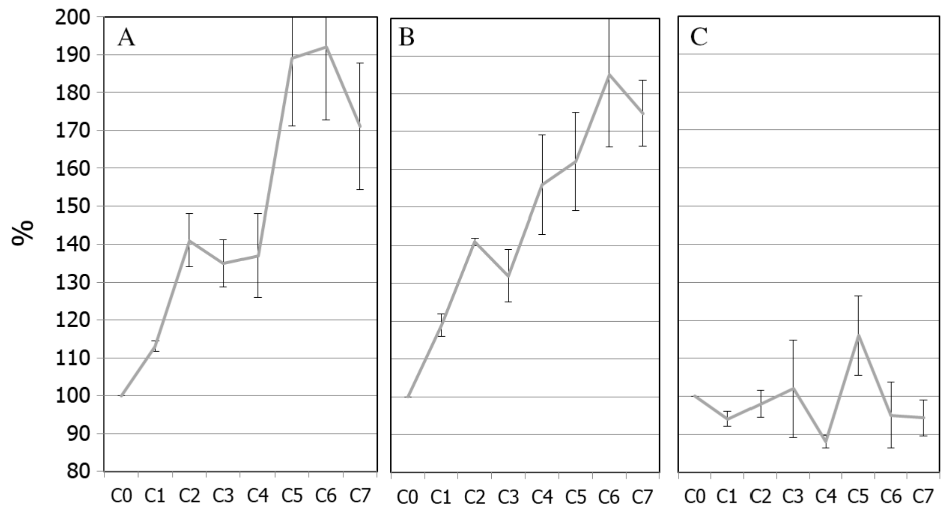


Table 1. Rubber yield, rubber percentage, root dry weight, selection differentials, standard deviations (SD) and coefficients of variation (CV) of Russian dandelion, cycle 4 (C4) to C7.

		Mean						
Year	Population	C0 control	Population[a]	Selected families	Selection differential[b]	SD	Selection differential/SD	CV (%)
Rubber yield (g·plant^{-1})								
2016	C4	0.257	0.342	0.457	0.115	0.084	1.36	25
2017	C5	0.199	0.377	0.578	0.201	0.118	1.71	31
2018	C6	0.136	0.217	0.336	0.119	0.083	1.44	38
2019	C7	0.145	0.240	0.344	0.104	0.068	1.54	28
Rubber (%)								
2016	C4	4.82	7.27	8.39	1.12	1.24	0.90	17
2017	C5	4.60	7.35	8.40	1.05	1.36	0.78	19
2018	C6	3.73	6.52	8.25	1.73	1.37	1.26	21
2019	C7	3.25	5.58	6.64	1.06	0.95	1.11	17
Root dry wt. (g·plant^{-1})								
2016	C4	5.33	4.66	5.44	0.78	0.80	0.98	17
2017	C5	4.36	4.99	6.95	1.96	1.33	1.47	27
2018	C6	3.37	3.08	3.93	0.85	0.82	1.03	27
2019	C7	4.22	4.00	4.91	0.91	0.77	1.18	19

Note: Populations grown at the Simcoe Research Station, Simcoe, ON, Canada, from 2016 to 2019.
[a]Population means were calculated in each year of the selection experiment for the respective populations. Means do not include data generated from the genetic gain experiment where C0, C4, and C8 populations were compared together at four sites in 2020.
[b]Selection differential = selected families mean — population mean.

received weekly trickle irrigation, indicating that soil structure and its ability to minimize leaching of fertilizer may be more critical than, or equally critical to, water holding capacity. The two sites at Woodstock, both on loam soils differed for root dry weight and survival. The location with decreased levels likely suffered drought stress from diminished sub-surface water availability compared with the other site. Consequently, site selection, soil type and water availability will greatly affect rubber yields.

Responses to selection for rubber yield and percentage and root dry weight, based on populations grown with a C0 control in separate years, were both positive and negative for individual cycles (Fig. 2). Since population means were based on two to four replicates of 100 to 193 families, combining 200 to 772 values, and C0 control means were estimated averaging two to eight values, decreased accuracy of the C0 means can significantly affect relative gain and explain the observations. Despite the variable responses, regression analysis of rubber yield and rubber percentage showed gains per cycle consistent with the 2020 data where the C0, C4 and C8 populations were grown in the same experiment.

Selection gain for rubber percentage and yield were approximately 10% per cycle when estimated from both the 2020 experiment and the analysis of specific populations in separate years. Consequently, population means can double after 10 cycles of selection. Continued gain is dependent on genetic variation, and coefficients of variation, assessing phenotypic variation among families in each population, were variable and did not show a consistent decreasing trend. Prior to C4, 100 families were grown and 10 selected. For C4, 20 of 90 families were selected and beginning with C5, 20 were selected from populations with 121 to 193 families. Thus, efforts to increase the number of selected families may have contributed to maintaining variation for selection.

Mean rubber percentage of the C8 population was 7.6% across the four sites, and varied from 6.7% to 8.4% among sites (data not shown). Early breeding efforts prior to World War II resulted in germplasm with 15% rubber (Koroleva 1940, as cited in Whaley and Bowen 1947). For individual roots in populations, 28% rubber has been reported (Lipschitz 1934, as cited in Krotkov 1945) presenting a promising prospect for long-term selection in the breeding population developed here.

Reports of high rubber percentages in individual roots, however, may not always reflect high rubber yields if root mass is low; a negative correlation has been reported between rubber percentage and root mass in some (Filippov 1941, as cited in Whaley and Bowen 1947) but not all experiments (Cornish et al. 2016; Hodgson-Kratky et al. 2017; Eggert et al. 2018). Selecting for rubber yield, accounting for both traits, rather than selecting for rubber percentage alone, can be critical for success. Correlated effects from selecting only for concentration with no control for organ size was observed with maize recurrent selection for oil percentage where high- and low-oil populations had decreased and increased kernel size, respectively (Woodworth et al. 1952).

The selection program originally bred populations for both sand and loam soils, growing two replicate blocks of each for evaluation. Beginning with the C4, selection continued only for the loam adapted population, with families evaluated in two blocks on each of two sites at the Simcoe Research Station to improve selection gain, especially for root dry weight. Despite the increasing replication, response to selection for this trait was not improved. With low heritability, additional replications, and most importantly, increased plot size may be necessary. In addition, improved harvesting equipment to minimize breakage and recover deep taproots may also be helpful. Without meaningful changes to estimates of root dry weight, selection gain for rubber yield will be limited by the ability to improve rubber percentage. Tysdal and Rands (1953) improved rubber yield by increasing both rubber percentage and plant dry weight for one cycle of polycross breeding where individual plants were selected and vegetatively propagated; this method, however, has limitations compared with recurrent selection.

The increased rubber yield of the C8 population can have an impact on the economic viability of Russian dandelion as a new crop. Bates et al. (2019) determined an optimal planting density of 4.94 million plants per hectare in outdoor planting boxes, where 900 kg·ha^{-1} rubber yield was estimated in unimproved germplasm six months after planting. Applying this density to the field data of C0 and C8 populations, rubber yields of approximately 1000 and 1800 kg·ha^{-1} are predicted, respectively. Although the response of these populations to high density under field conditions is unknown, the similarity of rubber yield between the data of Bates et al. (2019) and the C0 population is noteworthy. Comparatively, the rubber tree can produce rubber yields of 500 to 1500 kg·ha^{-1}·y^{-1} beginning 5 to 7 yr after planting (van Beilen and Poirier 2007).

Overall, half-sib family recurrent selection has been successful to improve both rubber yield and percentage in Russian dandelion. Gains averaging 10% per cycle have been achieved and continued advancements are possible as variation among families has not diminished with eight selection cycles. The crop is sensitive to soil type and drought which will impact where it may be grown, production costs and profitability. Despite success with breeding to improve rubber yield, additional research in weed control, direct seeding, seedling establishment and other agronomic practices are required for commercial production.

Competing Interests

The authors declare there are no competing interests.

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