

Back to perennials: Does selection enhance tradeoffs between yield and longevity?



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ABSTRACT

Perennial plants allocate more resources belowground and have longer-lived leaves than their annual counterparts, which are the basis for the promotion of perennial crops towards a more sustainable agriculture. On the downside, perennial plants selected for high seed-yield might show tradeoffs between current reproduction and long-term reproduction or survival, raising the questions of whether this high yield can be sustained over time and whether such tradeoffs can be overcome through selection. We compared growth, reproduction and the storage of reserves, over 3 years in wild and high-yielding accessions of *Physaria mendocina*. We found evidence of a tradeoff between current and future reproduction, responsible for a decrease in yield with age, in selected accessions. Selected accession had 76% more accumulated seed-yield in relation to wild accessions, but they also concentrated seed-yield and had an enhanced harvest index in the first year. Wild accessions maintained seed production over time. Resources allocated to reproduction were limited for both root allocation and carbohydrate accumulation after the second year. Wild and selected accessions consumed half of their storage carbon during reproduction, but replenishment of storage organs was 60% lower in selected accession compared to wild ones. This response could be related to a lower recovery of the root system after senescence. Empirical information about the pattern of use of reserves and their relationships with the ontogenetic changes in leaf functioning and root architecture is crucial when defining new criteria of selection of perennial species, since they will influence longevity and yield stability.

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1. Introduction

Agricultural systems around the world are facing a renewed demand to improve sustainability. Traditional systems relying on annual crops have substantial negative impacts on ecosystem functions (i.e. nutrient cycling, water quality and carbon emissions; Pimentel et al., 2012), leading to changes in dryland salinity, soil erosion and degradation, and nutrient leaching (Tilman et al., 2011; Pimentel et al., 2012). These problems can be reduced through the reintroduction in agricultural systems of productive and profitable perennial plants because they more closely mimic the original vegetation (Jackson, 2002; Ridley and Pannell, 2005; Cox et al., 2006; DeHaan et al., 2005; González-Paleo and Ravetta, 2015). Perennials have deep long-lived roots with a greater carbon storage capacity than annuals. This deep root facilitates water infiltration, reduces soil erosion risks, and maintains more soil carbon compared with annual crops (Glover and Reganold, 2010). Leaf tissues of perennials are usually more lignified to increase leaf persistence, tolerate

herbivory and resist environmental stress. Plants with long-lived leaves are more efficient in water and nutrients utilization (Wright et al., 2004), and need less fertilization because they can reutilize nutrients in following years.

In spite of this potential increased sustainability of perennial grain crop systems compared to that of traditional annual systems, the existence of tradeoffs between longevity and seed-yield still constitutes a major obstacle for extensive cultivation of perennials (Wagoner, 1990; Pimm, 1997; DeHaan et al., 2005). One potential approach to develop high-yielding perennial crops involves the conversion of promising wild perennials species into crops (Cox et al., 2002). In this case, the candidate is already perennial, but lacks most of the characteristics that would make it a good crop, and therefore, it needs to be selected for desirable agronomic traits, such as high seed production and harvest index, reduced fruit dehiscence, etc. (DeHaan et al., 2016). The challenge of breeding programs is to maintain those traits associated to the perennial habit (i.e. root system, storage capacity, leaf longevity, among others) while increasing yield and maintaining it stable for several years (Cox et al., 1985; Glover and Reganold, 2010; Vico et al., 2015).

Perennial species which have been selected for increased seed-yield usually show the cost of reproduction in terms of growth (i.e.

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reduced biomass production), sustained reproduction (i.e. lower seed-yield or number of fruits as years of cultivation progress), or survival (lower storage capacity; Piper and Kulakow, 1994; Aragón et al., 2009) in subsequent years. In spite of a general agreement in the ecological literature over the existence of trade-offs between current reproduction and future plant performance, empirical results are inconsistent. Some studies describe negative effects of current reproduction on future performance, while others fail to detect these negative effects (Obeso, 2002; Piper, 1993; Reekie and Avila-Sakar, 2005; Van Dijk, 2009). Plant breeders have pointed out that these physiological tradeoffs do not necessarily limit productivity of perennials because long-lived plants intercept more sunlight over the course of a year than their annual counterparts (e.g. Glover et al., 2010) or have an instantaneous net CO₂ uptake higher than co-generic annuals (Jaikumar et al., 2013). Therefore, at least some perennials appear to have enough total carbon available both for current reproduction and future performance. Even if these tradeoffs exist, crops can potentially be selected simultaneously for traits that increase seed-yield while maintaining plant longevity (Jackson and Dewald, 1994; DeHaan et al., 2005; DeHaan and Van Tassel, 2014).

Because of these functional uncertainties, there is a clear need for insight into the indirect changes provoked by selection for high yield on plants' future performance (growth, reproduction and survival), in order to identify additional selection criteria for wild perennials. Here we focused on two questions: Does increased yield have indirect costs on future growth, reproduction or longevity? Is the increased yield of selected perennial plants stable over time? We compared growth, reproduction and storage over time in wild and high-yield selected accessions of *Physaria mendocina* (Phil) Kurtz (Brassicaceae) to test the following hypotheses:

- 1) Selection for high seed-yield will provoke tradeoffs between current and future reproduction. We predict that reproduction (seed yield and yield components) will be maximum during the first year and will decrease with age (i.e. instability of yield) in selected accessions, while allocation to reproduction will be lower and more stable over time in wild plants.
- 2) Selection for high-yield will provoke tradeoffs between current reproduction and future growth. We predict that growth will be maximum during the first year and will decrease with age in selected accessions, while these traits will be more stable over time in wild plants.
- 3) Selection for high seed-yield will change the pattern of accumulation of total non-structural carbohydrates (TNC). We predict that wild accession will show a full recovery of root TNC levels after each reproductive event, while the regain of stores in high-yield accessions will be incomplete, leading to a decrease of the pool of TNC with age (i.e. tradeoffs between reproduction and longevity).

2. Materials and methods

2.1. Plant material and experimental design

Physaria mendocina (Brassicaceae) is a perennial forb, native to the Monte desert in Argentina. It forms an acaulescent rosette that develops elongate stems (bolting) bearing leaves, flowers and capsules during spring and early summer. The end of the reproductive period occurs in late January (González-Paleo and Ravetta, 2012). Plants reach 15–30 cm in height and produce many stems. Seeds are contained in pea-sized, bladder-like pods along the stem. The seed size is smaller than alfalfa and most clover species. All *Physaria* species evaluated so far are self-incompatible and cross pollinated (Dierig and Ray, 2008). This species, as well as other species within

the genus *Physaria* have been proposed as potential new seed-oil crops for drylands, with mediterranean-type climates (Dierig et al., 1993). In particular *P. mendocina* exhibits a conservative resource-use strategy that might contribute to increased ecological and economical sustainability in low-resource environments (Ravetta and Soriano, 1998; González-Paleo and Ravetta, 2011, 2015; Pastor-Pastor et al., 2015). *Physaria* seeds contain hydroxy fatty-acids, similar to those of castor oil (lesquerolic, auricolic and densipolic acids; Dierig et al., 1993). Large markets exist for these chemical feedstocks for lithium greases, polymers in paints and coatings, base stocks for lubricants, nylon, hydraulic fluids, and applications in the personal care industry. The hydroxyl group of these oils makes it a prime candidate as additive to diesel fuel to improve lubricity (Naughton, 1992).

A field experiment was carried out in Patagonia, Argentina (43°81'70" S; 65° 82'90" W). We compared wild and high-yield selected accessions in a completely randomized design. Seedlings of the wild accession came from seed collected from a native stand at Lihue-Calel, La Pampa, Argentina and multiplied in Chubut. This same wild accession was the source of base germplasm to generate the selected accession through mass selection (González-Paleo and Ravetta, 2012). The selection criterium was individual plant seed yield (bulk seeds of three top yielding plants). This criterium was applied to plants growing in stands of 160 plants per species, in introductory gardens in the Chubut River Valley. The selection cycle was carried on for three generations (González-Paleo and Ravetta, 2011).

Seeds of both groups were sown in the fall, in germination trays filled with soil: peat moss (1:1 by volume). Seedlings were transplanted to the field 66 days after sowing in a completely randomized experiment comparing selected types to wild types. Experimental units consisted in plots (2 m wide × 1.5 m long) with a density of 10 plants m⁻², with 0.17 m between plants. Rows were 0.4 m apart. Each plot had 32 plants, 10 reps per selection level, for a total of 320 plants per selection level. Plots were flood irrigated every 20–25 days until field capacity. Weeds were removed manually. Fungal controls with etilen bis ditiocarbamate-zinc (zineb, 2 g/l) and 2-metoxicarbamoil-bencimidazol (carbendazim, 0.5 cm³/l) were applied monthly.

2.2. Plant measurements

During three years, plants were harvested at three phenological stages: (1) rosette: vegetative stage before bolting; (2) anthesis: 50% of floral stems of a plant bear at least one flower; (3) maturity: 50% of floral stems of a plant bear at least one mature pod with dark and hard seeds.

We compared the dynamics of growth and reproduction of selected types and wild plants over three years. We used dry total biomass and leaf dry biomass as a proxy for growth (Poorter et al., 2009) and seed-yield and yield components as proxies for reproduction (Violle et al., 2007). Root allocation (dry mass) and carbohydrates reserves were used as a fitness proxy for survival and plant longevity (Kobe, 1997; Vilela and González-Paleo, 2015). We also compared the dynamics of reserves accumulation and use over time between wild and selected accessions.

For allocation analysis, individual plants (10 reps per selection group; 1 plant per plot) were separated into roots, leaves, and reproductive structures, and placed in an oven at 60 °C until weight constancy, and weighed. Total biomass included roots that were sampled using a core of similar diameter of the above-ground rosette. Seed-yield was determined at maturity (10 reps per selection group; 1 plant per plot). We counted the number of fruits per plant and the number of seeds per fruit. Individual seed-weight was estimated using sub-samples of 100 seeds. Harvest index (HI) was

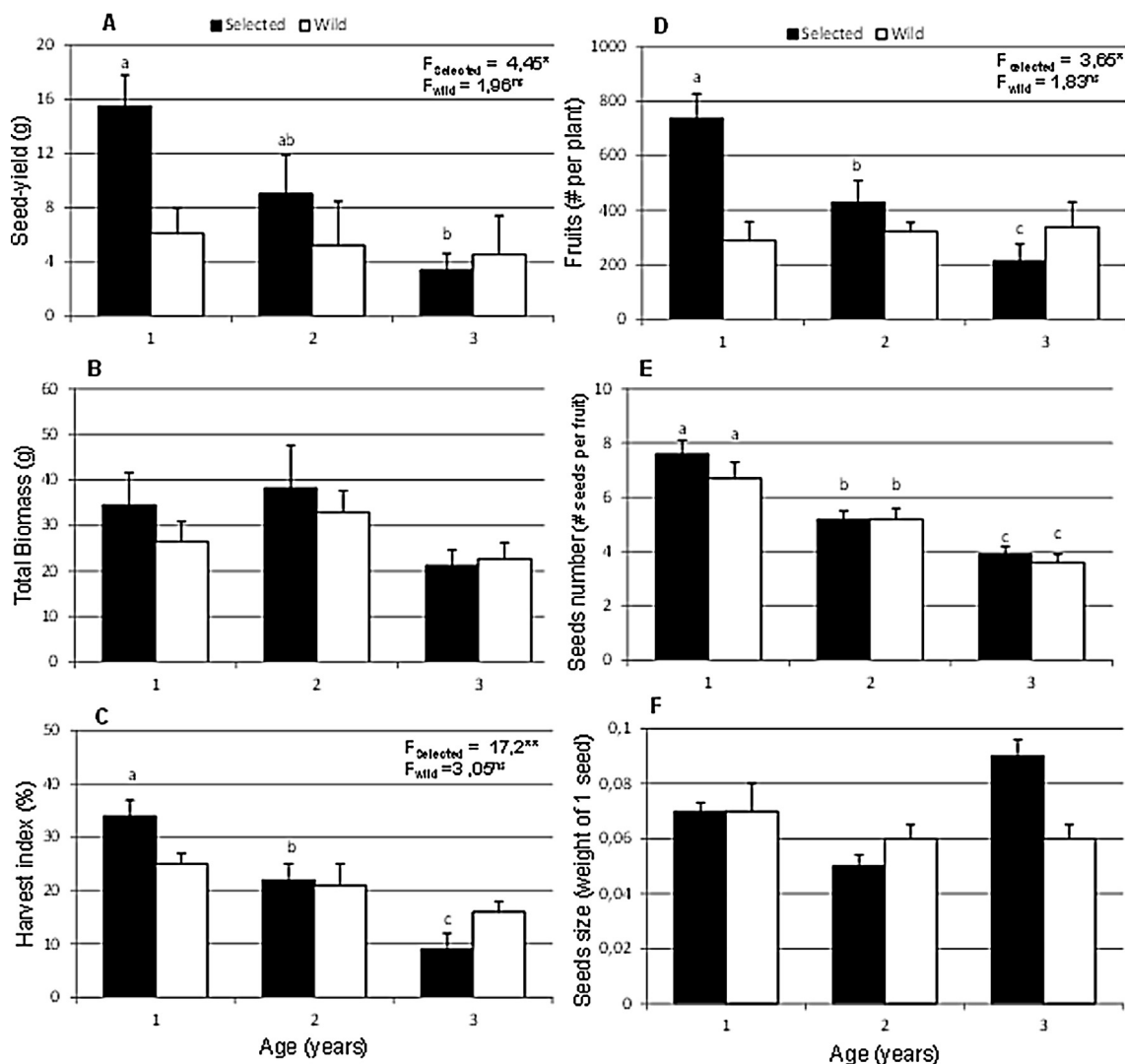


Fig. 1. (A) Seed-yield (g per plant), (B) total biomass (g dry weight per plant), (C) harvest index (% total dry weight), (D) fruits (number per plant), (E) seeds (number per fruit), and (F) seed size (weight of 1 seed), for 1-, 2- and 3-year old plants of selected and wild accessions of *Physaria mendocina*. Traits were measured at maturity. In (A), (C) and (D), different letters show significant differences between years (1, 2, and 3) for each accession (significant interaction Selection group * Age; Table 1). F values for differences among years for each accession are shown. In (B) and (F) there were no effects of any factor (Selection group and Age). For (E), different letters indicate Age effect for both Selection groups (non-significant interaction). *, $p < 0.05$; **, $p < 0.01$; ns, non-significant differences.

calculated as the ratio between seed yield and total dry biomass (including roots).

The concentration of total non-structural carbohydrates (TNC) was measured in roots at the rosette stage, anthesis, and maturity (6 reps per selection group; 1 plant per plot; 6 plots per selection group). Root dry-biomass (250 mg) was extracted with 70 ml of 10% (v/v) chlorhydric acid at 100°C for an hour. With this method the starch is hydrolyzed to glucose and incorporated to units of monosaccharides present in the sample. Glucose in the hydrolyzed extract can be determined colorimetrically using anthrone reagents, according to the method of Yemm and Willis (1954). The pool of non-structural carbohydrate (Pool TNC) was calculated as root TNC concentration * root mass (Kobe et al., 2010). TNC concentration and pool were measured in roots because this organ stores more TNC reserves than stems (Vilela and González-Paleo, 2015).

2.3. Statistical analyses

Biomass, seed-yield, yield components and TNC were measured over time on the same plots. We performed repeated measures

and used general linear model (GLM) in order to detect overall differences between selected and non-selected groups through time (plants of 1, 2, and 3 years-old). In those cases in which the interaction between selection group and age was significant, we checked for differences between wild and selected accessions at each age. We checked the trend over time for each group. Tukey's multiple comparison test was used for post hoc comparisons between wild and selected types.

3. Results

3.1. Effect of increased seed-yield on future growth and reproduction

In the perennial selected type, seed-yield, harvest index (HI) and the number of fruits per plant decreased as crop cycles progressed (Fig. 1). Maximum values of these traits were found in 1 year-old plants. Wild plants did not vary significantly over time for these traits (seed-yield, HI, and number of fruits per plant; Figs. 1A, C, and D). This difference in ontogenetic stability accounted for a significant interaction of age by selection group (Table 1).

Table 1

Repeated measures general linear model (GLM) for seed-yield and their components: total biomass, harvest index, number of fruits per plant, number of seed per fruits, and seed size. All traits were measured at maturity. n = 10. Statistics are F values. * p < 0.05; ** p < 0.01; *** p < 0.001; ns, non-significant.

	df	Seed-yield (g per plant)	Harvests index (%)	Total biomass (g per plant)	Fruits number (# per plant)	Seed number (# seed per fruit)	Seed size (g 1 seed)
Means squares							
Between Subjects							
Selection group	1	3.7 ^{ns}	0.01 ^{ns}	185.3 ^{ns}	211.4 ^{ns}	0.037 ^{ns}	0.002 ^{ns}
Error	18	103.2	0.02	611.5	201098.8	2.13	0.004
Within Subjects							
Age	2	244.4 ^{ns}	0.02 ^{ns}	1793.7 ^{ns}	366383.1 ^{ns}	75.5*	0.003 ^{ns}
Age × Selection group	2	320.5*	0.23**	1148.3 ^{ns}	803143.5*	0.45 ns	0.002 ^{ns}
Error	36	104.5	0.02 ^{ns}	518.8	25.3516.1	1.59	0.004

Bold values are significant effects in de GLM.

Irrespective of selection group and age, total biomass and seed-size were stable over time (Table 1; Figs. 1B and F) and the number of seeds per fruit decreased with age (Table 1, Fig. 1E).

Differences between wild and selected accessions in seed yield, HI, and number of fruits were only significant during the first reproductive event ($F = 6.1^{***}$, $F = 3.86^*$ and $F = 5.93^{**}$, respectively). Wild and selected 2 and 3 years-old plants showed no significant difference in these traits (Fig. 1).

Biomass allocation to leaves increased during the first year. Leaf biomass decreased during the reproductive period (from rosette to maturity) and recovered during the vegetative period (from maturity to next-year rosette; Fig. 2a). This pattern did not differ between selection groups (non-significant interaction; Table 2).

3.2. Effect of increased seed-yield on the pattern of carbon storage

Biomass allocation to roots over time differed between groups (significant interaction age x selection group; Table 2). In both

groups root biomass increased from 1st to 2nd year rosette and then decreased during the 2nd reproductive period (from 2nd rosette to 2nd maturity). After that, wild plants gained root biomass (from 2nd maturity to 3rd rosette) while in selected plants root biomass remained stable during the 3rd year (Fig. 2b).

The concentration of total non-structural carbohydrates in the roots (TNC) was always lower in selected accessions than in their wild relatives (Table 2, Fig. 3a). The ontogenetic pattern of accumulation and use of the pool of TNC differed between selection groups (Table 2). During the first year, the pool increased moderately from rosette to maturity in both groups. During the 2nd and 3rd year, the greatest accumulation of TNC occurred from the end of the reproductive period (maturity) to the beginning of the following reproductive episode (rosette). During this recovery period, wild plants increased almost 3-fold their pool of reserves the 2nd year and 2-fold the 3rd year. The increment in TNC from maturity to rosette was more modest in selected plants: 120% and 12% for the 2nd and 3rd year, respectively (Fig. 3b).

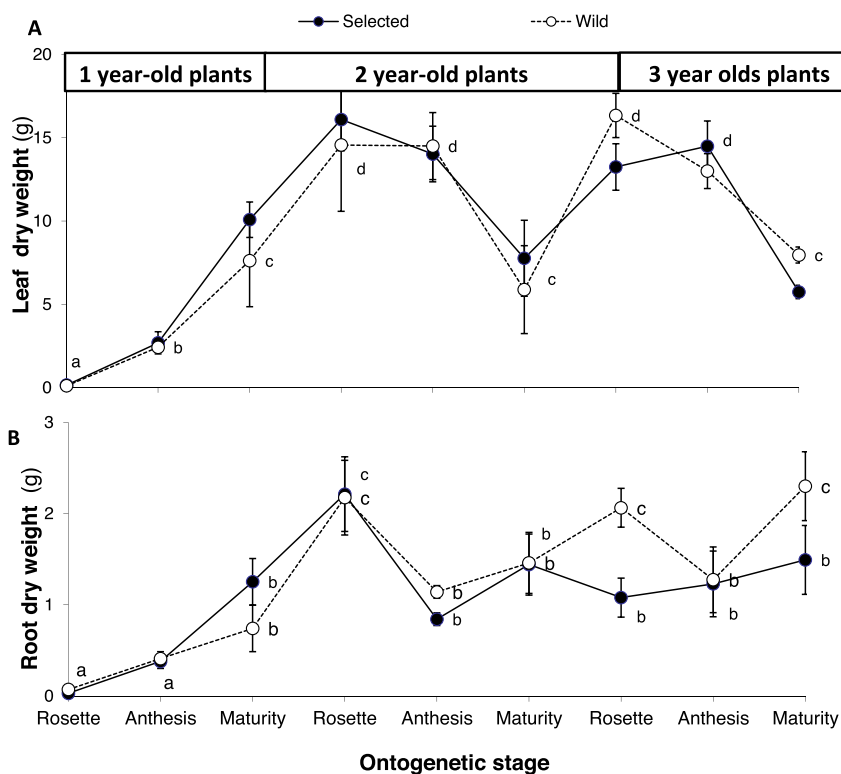


Fig. 2. Ontogenetic pattern for three years of A) leaf (g per plant) and B) root (g per plant) allocation, for Selected and Wild accessions of *Physaria mendocina*. In (A) different letters indicate differences among ontogenetic stages (Age effect) for both accessions (non-significant interaction Selection group × Age, Table 2). In (B) letters show differences among ontogenetic stages for each accession (significant interaction Selection group × Age, Table 2).

Table 2
Repeated measure general linear model (GLM) for biomass components: root and leaf allocation, and storage: total non-structural carbohydrates (TNC) concentration and PoolTNC. All traits were measured at rosette, anthesis and maturity of 1-, 2-, and 3-year old plants. n = 10; ^a n = 6. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, non-significant.

	Leaves (dry weight, g)		Roots (dry weight, g)		TNC ^a (% of root dry weight)		Pool TNC ^a (mg TNC)	
	df	MS	df	MS	df	MS	df	MS
Between Subjects								
Selection groups	1	8.17 ^{ns}	1	0.36 ^{ns}	1	1057.9***	1	94.7**
Error	18	83.12	18	2.15	10	15.39	10	46.23
Within Subjects								
Age	8	223.61***	8	8.22***	8	173.3***	8	85.14***
Age × Selection groups	8	43.73 ^{ns}	8	15.25***	8	54.5***	8	59.95**
Error	144	23.04	144	2.68	80	18.47	80	16.34

Bold values are significant effects in de GLM.

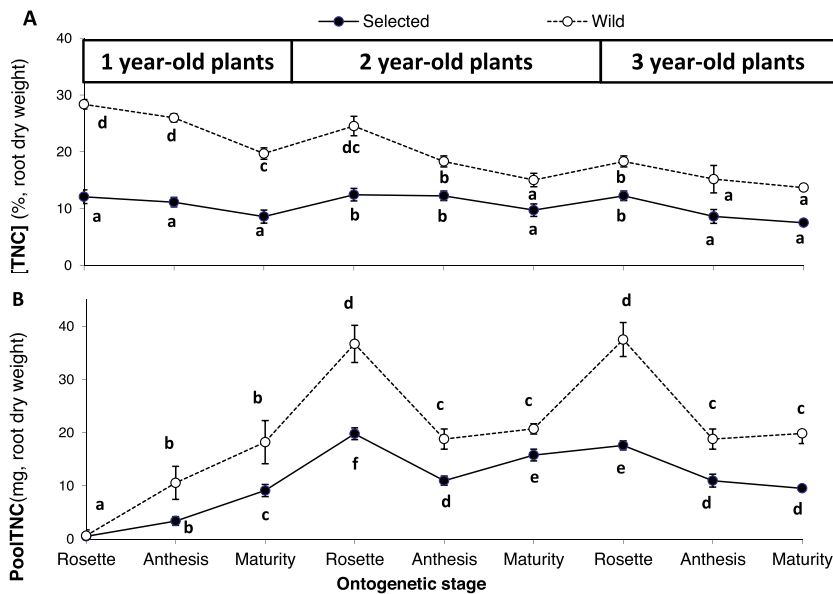


Fig. 3. Ontogenetic pattern for three years of (A) TNC concentration (total non-structural carbohydrates; % root dry weight) and (B) PoolTNC (mg of root dry weight) for Selected and Wild accessions of *Physaria mendocina*. Different letters indicate differences among ontogenetic stages (Age effect) for each accession (significant interaction Selection group × Age, Table 2).

In both groups, reproduction in the 2nd and 3rd year triggered the depletion of reserves by half, (from rosette to anthesis; Fig. 3b). At the end of the three years, the pool of TNC of wild plants was twice as large as that of selected plants (20 g vs 10 g, respectively; Fig. 3b).

4. Discussion

Perennial grains are being developed because of their potential to improved ecosystem services when cultivated (i.e. Crews, 2005), and potentially lower production costs. The breeding process for almost every crop involves direct selection for plant productivity (i.e. seed- yield), irrespective of the life cycle of the plant (annual or perennial; Cox et al., 2002). This type of selection criteria has been found to promote a number of additional changes in plant phenotypes such as days to anthesis, fruit dehiscence, allocation to belowground organs, and leaf morphology (Evans, 1993; Doebley et al., 2006; González-Paleo and Ravetta, 2011, 2012; Vilela and González-Paleo, 2015). Reductions in pest resistance and drought tolerance have also been found when selecting annual species (Denison, 2012; Hancock, 2005; García-Palacios et al., 2013). However, there is little information on the effect that selection exerts on future growth, reproduction and longevity in perennials (Jaikumar et al., 2013; Vico et al., 2015). Most experimental studies have

addressed the question of seed-yield versus persistence over seasons only indirectly (Jackson and Jackson, 1999; González-Paleo and Ravetta, 2011). Here we checked for the existence of trade-offs between longevity, current and future reproduction and growth in *Physaria mendocina*, a potential new oil-seed crop for arid and Mediterranean environments.

Our first question inquired whether the increase in seed-yield generated by selection would be sustained over several growing seasons. Accessions of *Physaria mendocina*, for which seed-yield was the selection criteria, had an increase of 76% in accumulated seed-yield over three years, compared to wild accession (i.e. sum of seed-yield of the first three reproductive events), but selection also changed yield distribution over time, by concentrating yield on the first year. Sixty percent of the total accumulated seed-yield was produced in the first reproductive event. This change in seed-yield distribution was associated to an initial increase of harvest index and number of fruits per plant, which was not sustained in subsequent years. These results support the existence of trade-offs between current and future reproduction. Yield was higher in selected plants only during the first year. Wild and selected plants of 2- and 3-years showed similar seed-yield. Similar responses have been found in the past with at least another perennial crop; for example, 25 years of work with perennial wheat (*Triticum aestivum* hybridized with perennial grasses) had produced lines whose

first-year yields are economically profitable, but with a rapid yield decline in subsequent years (Suneson et al., 1963).

The change in the distribution of seed-yield over the years provoked by selection found in other crops, has been related to the pattern of allocation of the pool of available meristems (Clarke et al., 2013). It has been suggested that in plants with a limited numbers of meristems, if a higher proportion of it differentiate into flowers in the first reproductive event, only a limited number of meristems remain to contribute to future reproduction (Geber, 1990; Huber and During, 2001). In the case of *Physaria*, the cause of the decay in yield through the years is not known and needs to be tested.

The change in the distribution of seed-yield was not accompanied by a change in growth over time. Despite our prediction that total biomass and leaf biomass would be at a maximum during the first year and would decrease with age in selected plants, we were not able to find evidence of trade-offs between current reproduction and future growth. Wild and selected plants did not differ in growth. In other perennial rosette plants it has also been reported that reproductive allocation does not tradeoff with growth (Vilela et al., 2008a) therefore a larger biomass could be used to increase yield (Vilela et al., 2008b). Alternative explanations for this lack of trade-off are factors that might make the cost of reproduction relatively small or driven by factors other than direct resource competition with growth. Reproductive structures may supply a large part of the resources required, or photosynthetic rates may increase if the demand for carbon increases during reproduction (Lambers et al., 1998; Ploschuk et al., 2005).

Stored carbohydrate reserves can provide an alternative source of C for reproduction (i.e. bolting, seed-filling, etc.) when photosynthetic capacity is reduced during periods of environmental and biotic stresses (Janecek et al., 2015) or at the beginning of the annual growth-cycle. Depletion of carbohydrate storage is often associated with a lower survival (Myers and Kitajima, 2007; Piper et al., 2009; Vilela and González-Paleo, 2015), lower capacity for regrowth (Vilela et al., 2012, 2016), lower resistance to biotic attack, and a less plant longevity (Chiarello and Roughgarden, 1994; González Paleo and Ravetta, 2011). Results about the existence of trade-offs between reproduction and longevity are contradictory in perennial species (Jackson and DeWald, 1994; Piper and Kulakow, 1994; Cox et al., 2006; DeHaan and Van Tassel, 2014). We are not aware of any work dealing with the negative effect of increased seed-yield on future stored reserves. Our results support the proposal that there is competition between different carbon sinks: biomass allocation to seeds and reproductive structures limited both root allocation and total non-structural carbohydrates accumulation after the second year. This tradeoff between storage and reproduction challenges the feasibility of domesticating perennial grains (Denison, 2012).

In Mediterranean-type environments, reproductive events of perennial species are followed by a dormant period (Latzel et al., 2014). This dormant period allows the recovery of root systems and leaf re-sprouting, both processes that enable refilling of storage organs (Vriet et al., 2014). Winter dormancy is followed by a reproductive period characterized by carbohydrate consumption. Wild and selected accessions of *Physaria* differed in their storage dynamics during the dormant and reproductive periods. Therefore, selection for increased seed-yield changed the pattern of accumulation of TNC. From 1st year to 2nd year rosette, both groups of plants increased biomass allocation to roots and their pool of carbohydrates. During the second reproductive period, both groups consumed about 50% of their stored carbohydrates. However, refilling of storage organs was 60% lower in the selected accession compared to the wild one, and after three years resulted in a reduction of 50% in the pool of TNCs in selected accession. This response was caused by a lower recovery of their root biomass after senescence, rather than by differences in the pattern of TNCs concentration. It has been shown that high allocation to below-

ground organs in perennials during their first growing season is not changed during artificial selection (Vico et al., 2015). However, in our experiment, the cost of increased seed production on biomass allocation to roots and carbohydrates was evident only in 2- and 3 year-old plants. These results suggest that after selection for yield, perennial plants may not be able to maintain these root traits, and thus, their regulatory ecosystem services. Enhanced sustainability may not hold, as expected for perennial crops.

Besides the consequences of changes in partition in the recovery of the stored carbon levels, leaf traits such as specific leaf area, leaf longevity, the rate of senescence, among others, are responsible for known differences in carbon gain and further storage (Hernández et al., 2011; Jaikumar et al., 2013). In several species of *Physaria*, we have found that selection for increased yield decreased leaf longevity and photosynthetic capacity and increased senescence, which was also related to an increase in specific leaf area (SLA), one of the main indirect changes provoked by artificial selection for increased seed-yield in *Physaria* (González-Paleo and Ravetta, 2011). Taken all together these structural and functional changes could explain the incomplete recovery of the root system and TNC pool, and in the long term, should reduce plant longevity.

Despite the important roles identified for stored carbohydrates, little is known about their pattern of accumulation and which are the controls that regulate their mobilization into growth or reproduction. Is carbohydrate storage a passive process that accumulates only when C is in excess and indicates the status of the plant's C balances (Körner, 2003)? Or, is an active process that accumulates carbon at expense of competing sinks (Sala et al., 2012)? How much of the carbohydrate stored is available to be diverted to seed, and how much is sequestered permanently and accounts as a threshold to ensure plant longevity?. These are questions that are central to the development of industrial perennial crops.

Persistence and maintenance of seed-yield over seasons has been difficult to achieve in the past, and will occupy much of the attention of perennial-grain breeders (Cox et al., 2002; DeHaan et al., 2016). Empirical information about the pattern of accumulation and depletion of stored reserves, and their relationships with the ontogenetic changes in leaf functioning (i.e. C economy and N use efficiency) and root architecture, is crucial when defining new criteria of selection of perennial species, due to their associations with plant longevity and seed-yield stability.

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