Unpredicted, rapid and unintended structural and functional changes occurred during early domestication of Silphium integrifolium, a perennial oilseed

1 **Unpredicted, rapid and unintended structural and functional changes occurred during early domestication of**
2 ***Silphium integrifolium*, a perennial oilseed**
3
4 D. A. RAVETTA[*,+], A. E. VILELA[*], L. GONZALEZ PALEO[*], D. VAN TASSEL[**].
5
6 *CONICET, Museo Egidio Feruglio, Fontana 144 Trelew (9100), Chubut, Argentina[*]*
7 **The Land Institute, 2440 E Water Well Rd, Salina 67401, KS, USA[**]*
8
9 [+] *Author for Correspondence: ravetta@agro.uba.ar*
10
11 Running head: Early signs of crop evolution in perennial sunflower
12
13
14 **MAIN CONCLUSION**
15 Selection for increased yield changed structure, physiology and overall resource-use strategy from conservative
16 towards acquisitive leaves. Alternative criteria can be considered, to increase yield with less potentially negative
17 traits.
18
19 **ABSTRACT**
20
21 We compared the morphology, anatomy and physiology of wild and semi-domesticated (SD) accessions of *Silphium*
22 *integrifolium* (Asteraceae), in multi-year experiments. We hypothesized that several cycles of selection for seed-
23 yield would result in acquisitive leaves, including changes predicted by the leaf economic spectrum. Early-selection
24 indirectly resulted in leaf structural and functional changes. Leaf anatomy changed, increasing mesophyll
25 conductance and the size of xylem vessels and mesophyll cells increased. Leaves of SD plants were larger, heavier,
26 with lower stomatal conductance, lower internal CO_2 concentration, and lower resin concentration than those of wild
27 types. Despite increased water use efficiency, SD plants transpired 25% more because their increase in leaf area.
28 Unintended and undesired changes in functional plant traits could quickly become fixed during domestication,
29 shortening the lifespan and increasing resource consumption of the crop as well as having consequences in the
30 provision and regulation of ecosystem services.
31
32 **KEYWORDS**: A/Ci, Chlorophyll fluorescence; Gas exchange; Leaf anatomy; Mesophyll conductance; Net CO_2
33 uptake.
34

35 **Abbreviations**

36 A: Instantaneous CO_2 uptake [$\mu mol\ m^{-2}\ s^{-1}$]

37 C_i: Internal CO_2 concentration [$\mu mol\ mol^{-1}$]

38 E: Instantaneous transpiration rate [$mmol\ m^{-2}\ s^{-1}$]

39 gm: Mesophyll conductance [$\mu mol\ m^{-2}\ s{-1}\ Pa^{-1}$]

40 gs: Stomatal conductance [$mol\ m^{-2}\ s^{-1}$]

41 J: Electron transport rate [$\mu mol\ CO_2\ m^{-2}\ s^{-1}$]

42 SD: Semi-domesticated plant

43 SLA: Specific leaf area [$cm^2\ g^{-1}$]

44   TPU: Triose phosphate utilization [$\mu mol\ CO_2\ m^{-2}\ s^{-1}$]

45   Vcmax: Maximum RuBP carboxylation capacity [$\mu mol\ m^{-2}\ s^{-1}\ CO_2$]

46   W: Wild plant

47   Γ: Carbon compensation point [$\mu mol\ mol^{-1}$]

48

49

50   **INTRODUCTION**

51   Annual crops share a set of traits that have collectively been described as the "domestication syndrome".
52   Compared to their wild ancestors, crop plants are larger, more palatable, with larger seeds and leaves, and
53   indehiscent and non-toxic fruits (Evans 1993; Diamond 2002). For most annual crops, these changes were brought
54   about early in the domestication process and are different from those made by recent crop improvement that resulted
55   in modern cultivars (Milla et al. 2014). During this process, plants were moved from relatively high stress
56   environments to lower stress agricultural ecosystems, where interspecific plant competition and herbivory pressure
57   were reduced by human intervention, which, together with fertilization, burning and tillage also increased resource
58   availability (Connor et al. 2011). The release of natural stressful conditions should have resulted, in most annual
59   crops, in the shift of strategy of resource acquisition and use, from conservative to more acquisitive (Vilela and
60   Gonzalez-Paleo 2015), although there is evidence that this change may not be ubiquitous (Milla et al. 2014).
61   At a broad scale of analysis, it is known that leaf structural and functional traits are key characters in
62   describing resource use strategies. In general, leaves from acquisitive plants are thinner and less costly, have more
63   nitrogen and phosphorus per unit weight, and higher rates of assimilation and respiration (Wright et al. 2004; Diaz et
64   al. 2004, 2016). Following these general trends, it was predicted that leaves of domesticated plants should have
65   higher specific leaf area (SLA) than their wild counterparts, although Milla et al. (2014) found no evidence of this
66   change in a group of 30 annual crops: while leaf size increased, SLA did not change. Furthermore, leaf size mostly
67   increased during early domestication (from wild, undomesticated types to landraces) but not with modern breeding.
68   Leaf function has also been predicted to change during crop evolution (Pujol et al. 2008; Donovan et al.
69   2011; Jaikumar et al. 2013) because of the need to produce more carbohydrates to supply increased sink demand
70   (e.g., larger seed number and size, changes in seed composition, etc.) in higher yielding plants. Although
71   photosynthesis has been recognized as an option to achieve increased yields, the efforts to improve CO_2 assimilation
72   through genetic manipulation in C3 crops have had very limited success during the last two decades (Long et al.
73   2006; Flexas 2016). Photosynthetic rates have been found to remain unchanged after domestication in at least three
74   grain-crops (sunflower, maize and wheat; Gifford and Evans 1981; Milla and Matezanz 2017). In these crops,
75   increase in whole-plant C gains was explained by a larger leaf area per plant (i.e., leaf size increase) that resulted in
76   more transpiration and higher water footprint. While according to these authors, leaf photosynthesis remained
77   unchanged, manipulation of leaf anatomy may be an option to increase total photosynthesis without enlarging plant
78   size that has received little attention. For example, Tholen et al. (2012) listed a number of ideal leaf anatomical
79   features to favor CO_2 uptake under different environmental conditions, including larger veins, elongated palisade
80   cells and bundle sheath extensions to maintain the high hydraulic conductance required to improve photosynthesis.
81   Most domesticated plants have also lost chemical defenses either by direct selection against bitter or toxic
82   compounds (Gepts 2004; Meyer et al. 2012) or indirectly by shifts in resource allocation brought about by a change
83   in resource acquisition strategies (Mondolot et al. 2008). Low resource availability limits the rate at which resources
84   can be acquired (Coley et al. 1985), and fitness is maximized by protecting acquired resources (i.e., conservative
85   strategy). In contrast, in resource-rich environments acquisitive strategies are favored: plants acquire resources
86   rapidly and use them for growth and reproduction (Reich et al. 1997; Reich 1998; Poorter and De Jong 1999;
87   Poorter and Garnier 1999; Westoby et al. 2002). Under these conditions, regrowth after herbivory is a more cost-
88   effective strategy than the investment of carbon in defenses for the standing biomass (Poorter and Garnier 1999).

89 Contrasting domesticated plants and their wild counterparts, it has been shown that defensive chemistry of cultivars
90 has been drastically reduced as a result of breeding (Gols et al. 2008; Mondolot et al. 2008; Chacón-Fuentes et al.
91 2015; Whitehead et al. 2017). Meyer et al. (2012) showed that a decrease in plant secondary metabolites is the most
92 common domestication trait over a period of 7000 years.

93   Another feature lost by many grain crops in the process of domestication is the capacity to perenniate. Despite
94 the fact that many of the wild ancestors of several major crops such as maize, rice and sorghum were perennials (i.e.,
95 *Oryza rufipogon* and *Zea perennis*), only annual grain crops dominate the earth since the time of the earliest farmers
96 (Cox et al. 2002). One hypothesis is that perennial crops are constrained by a vegetative-reproductive tradeoff where
97 high reproductive allocation and sufficient storage allocation for perennation cannot coexist (Van Tassel et al. 2010).
98 In other words, it may be possible for artificial selection to drive increases in seed yield in wild, herbaceous
99 perennial species, but those increases may cause losses in allocation to vegetative and perennating structures,
100 resulting in a shift from perenniality to annuality (Denison 2012). Some studies have supported such tradeoffs (e.g.,
101 González-Paleo et al. 2016; Vico et al. 2016; Pastor-Pastor et al. 2018). Still, because of the ecological benefits of
102 cultivating perennials, in recent years there has been an increased interest in developing perennial grain crops (Cox
103 et al. 2002, 2006; DeHaan et al. 2005; Glover and Reganold 2010; Gonzalez-Paleo and Ravetta 2011; Pimentel et al.
104 2012; Kantar et al. 2016). Perennial relatives of sunflowers, wheat, rice, and sorghum, and specialty oilseeds are in
105 the process of domestication, either directly or through hybridization with the annual crop species (Ravetta and
106 Soriano 1998; Cox et al. 2002).

107   While the main advantage of the perennial habit is soil conservation due to the reduced need for tillage or
108 fallow periods in which the soil is exposed and vulnerable to erosion, the contrasting histories of natural selection
109 imply that perennial relatives should have evolved more conservative strategies than their annual counterparts at
110 many levels of organization beyond those involved in overwintering (Pimentel et al. 2012). Plants with structural
111 and functional traits enabling more conservative use of water and nitrogen and greater defenses could require lower
112 rates of agricultural inputs and should provide enhanced regulatory services and internal ecosystem regulation
113 compared to annual crops. There is recent evidence to support this proposal (Pastor-Pastor et al. 2018a; Gonzalez-
114 Paleo et al. 2019), although there is also evidence that selection for yield may quickly change perennial plants
115 towards the acquisitive side of the spectrum (Pastor-Pastor et al. 2018b).

116   New domesticates provide a unique opportunity to understand crop evolution and the transition from
117 perenniality to annuality undergone by our major grain crops. Within this general framework our objective is to
118 characterize potential changes in leaf structure and function brought about by domestication of a new perennial
119 oilseed crop. We use as a model *Silphium integrifolium* (Michaux, Asteraceae, rosinweed or silflower), a perennial
120 native to Eastern and Central United States. Silflower plants from semi-domesticated populations were larger and
121 had higher seed production than plants from wild populations growing in the same common garden (Vilela et al.
122 2018). Seed-yield increased more than biomass during early domestication, meaning that harvest index increased.
123 Similar changes have been found in annual sunflower (Milla and Matesanz 2017) and other crops after
124 domestication (Harlan 1973; Zohary 2004), although the changes reported in most previous studies are the
125 accumulated consequences of many years of unconscious and conscious selection, compared to what we found with
126 5 cycles of directed selection. We hypothesize that artificial selection using enhanced seed-yield as criterion during
127 the domestication of a wild perennial, should rapidly result in leaf structural and functional changes that would
128 move the plant phenotype, towards the acquisitive end of the leaf economic spectrum, even when the deliberate
129 selection completely ignores leaf traits.

130   At the morphological level, we predict that leaves from plants selected for enhanced seed-yield (SD) will be
131 larger, thinner, and with higher SLA than their wild, unselected counterparts (W). At the anatomical level, we
132 predict that leaves from SD will have larger mesophyll cells, a trait related to increased surface exposed to
133 intercellular air space, larger xylem vessels to maintain high stomata conductance and fewer resin ducts,
134 concomitant to a decrease in the investment of resources to defense secondary metabolites. At the physiological
135 level, these anatomical changes should result in higher overall conductance to water and CO_2, more CO_2 uptake, and
136 more transpiration.

137 To test these predictions, we used the long-lived perennial *Silphium integrifolium* as our experimental system
138 and compared in a multi-year field experiment and additional controlled experiments, wild accessions and semi-
139 domesticated breeding populations developed through five cycles of recurrent selection for increased seeds per
140 capitulum and other components of seed yield potential.
141
142 **MATERIALS AND METHODS**
143 **Plant material and study site**
144 *Silphium integrifolium* is an herbaceous, long-lived diploid (2n=2x=14), indigenous to central United
145 States. A full description of W and SD populations at The Land Institute, as well as the process of breeding has been
146 published in Vilela et al. (2018). Briefly, *Silphium* seed was harvested from wild populations in central KS in 2002.
147 Selections from the initial evaluation nursery were intermated to produce Cycle 1 plants. Selections from Cycles 1, 2
148 and 3 were intermated in 2006, 2009 and 2012, respectively. The general method has been to plant spaced-plant
149 evaluation nurseries, select 20-50 individual plants using information about the performance of each plant's half
150 siblings in the second year, dig the selected plants early in the third year and polycross them in a crossing nursery
151 (Van Tassel et al. 2014).
152 **Experiment 1: Wild vs Semi-Domesticated**
153 Research plots were located at The Land Institute's experiment station near Salina, Kansas (38°46'14"N
154 97°35'30" W). Soil types on the research farm include Hord silt loam (fine−silty, mixed, superactive, mesic Cumulic
155 Haplustolls) and Cozad silt loam (coarse−silty, mixed, superactive, mesic Typic Haplustolls). The location is 370 m
156 above sea level on the western fringe of the tallgrass prairie region. Mean annual precipitation in the area is 737 mm,
157 with an average of approximately one-tenth of that precipitation coming in the form of snow. Rainfall is
158 concentrated in spring and fall, and extended droughts are common in summer. The mean daily high temperature in
159 July is 34 ∘C, the mean nightly low in January is −7 ∘C, and rapid, wide swings in temperature are common in all
160 seasons.
161 Semi-Domesticated genotypes: One hundred and twenty plants from a breeding nursery were identified for
162 their good establishment and approximately equal representation from several half sib families. The breeding
163 nursery was established in the field in late May 2015 by transplanting approximately 1500, 8-week-old seedlings.
164 Spacing was 1m within in rows spaced 2 m apart such as to avoid competition. Transplants received additional water
165 (aprox. 0.5 l per plants) at the time of transplanting, with no additional irrigation other than rainfall. Weeds were
166 controlled using mechanical tillage and hand pulling. No fertilizer was applied until the spring of 2018 when urea
167 was broadcast in early May (56 kg/ha) in 2018 and 2019 because the grass and *Silphium* appeared nitrogen limited.
168 Wild genotypes: individual plants from nearby germplasm evaluation plots (820 plants total) were used for
169 comparison. These plots were of a similar age and managed similarly to the main experimental plot. The seeds used
170 to establish these plots were collected in 2013 from 12 estates, but only those wild populations coming from Kansas
171 were used for Experiment 1 (see complete list of populations in S1 of Vilela et al. 2018).
172
173 **Experiment 2: Cloned genotypes**
174 Eight genotypes from SD plants from Experiment 1 were selected, four having the highest Net CO_2 uptake
175 and four from the lowest Net CO_2 uptake rates. These eight genotypes were cloned. To generate these clonal
176 individuals, the dormant mother plants were dug up in early March 2019 and the crown and basal roots divided into
177 5-6 pieces. These pieces were planted in pots and grown in an unheated greenhouse in 4-gallon pots. At the time of
178 measurements, 4 months after transplanting, these individuals were bolting.
179 Details of experiments, *Silphium integrifolium* accessions, measurements, etc., can be graphically
180 visualized in Suppl. Fig. S1
181
182 **Measurements**
183 **Gas exchange**

184 Gas exchange was measured on all plants in Experiment 1 using a LICOR LI -6800 Portable
185 Photosynthesis system. On each of more than 100 SD plants, and 20 individual plants from W accessions,
186 instantaneous gas-exchange was measured on 3 leaves and averaged for a single value per plant, for each variable:
187 CO_2 uptake (A); transpiration rate (E); internal CO_2 concentration (C_i), and stomatal conductance (g_s).
188 Measurements were taken around noon (11:00 to 14:30) on fully expanded leaves, and exposed to full sun, and on
189 two or three consecutive dates (to accommodate all measurements). Measurements were taken in 2016, 2017, and
190 2018 on the same plants twice each year, during bolting and flowering stages (6-7-8 May 2017 and 11-12 June
191 2017; 17 May 2018 and 12 -13 June 2018). After a first year of gas exchange data collection, 8 individual plants
192 Semi-Domesticated were chosen: four for their high Net CO_2 uptake (High A, top 8% of the population) and four for
193 their low Net CO_2 uptake (Low A, bottom 8% of the population).
194 High A and Low A plants were selected for further characterization of gas exchange. Daily integrated CO_2
195 uptake ($A_{integrated}$) and transpiration ($E_{integrated}$) was estimated both in 2017 (12 May) and 2018 (21 June). Gas
196 exchange was measured 7 times, on 8 leaves per plant from sunrise to sunset to develop a daily march of CO_2 uptake
197 and water loss. The instantaneous values were then integrated to estimate daily and morning (sunrise to 1PM) totals
198 of $A_{integrated}$ and $E_{integrated}$.
199

200 **Internal CO_2 concentration (Ci) curves and fluorimetry**
201 To quantify biochemical processes limiting photosynthesis, A/Ci curves were generated (Farquhar et al.
202 1980) for High A and Low A plants in Experiment 1, in 2018 and 2019. From these curves 7 parameters were
203 estimated: ribulose bis-phosphate carboxylation capacity (Vc), electron transport rate (J), triose phosphate utilization
204 (TPU), carbon compensation point (Γ), and degree of stomatal limitation (Ls), CO_2 compensation point (R_d), and
205 mesophyll conductance (g_m). These parameters were estimated following Sharkey et al. (2007) and using the
206 calculator available at Sharkey 2016). These parameters were estimated using A at external CO_2 concentrations (Ca)
207 = 400, 300, 200, 100, 50, 10, 400, 400, 600, 800, 1000, 1200, and 1500 ppm (Long and Bernacchi 2003). These
208 measurements were taken on June 29, 2018 when plants were at the early flowering stage, in Salina, Kansas. On
209 each plant, curves were generated using the average instantaneous gas exchange of three leaves per plant. Measured
210 plants were growing in the field. Measurements were done, between 10:30 am and 3:00 pm, under controlled
211 chamber conditions of 2000 μmol CO_2 m^{-2} s^{-1} PPFD and 50% humidity. Leaf chamber temperature was 27°C.
212 On May 22, 2019, a new set of A/Ci curves was generated using clonal individuals in the Experiment 2.
213

214 **Leaf morphology**
215 Fresh leaf thickness was measured using a Mitutoyo Digital Thickness Gage 547-30 (3 repetitions/plant) in
216 SD and W genotypes in Experiment 1. Individual leaves (3 repetitions/plant) were harvested, and immediately
217 transported into the lab in sealed plastic bags. The position of the harvested leaves was the same in every plant: the
218 last completely expanded leaf and the ones immediately below. Leaves were weighed, scanned for the
219 determination of leaf area, oven-dried at 40°C for 72 h and weighed. Specific Leaf Area was estimated as the
220 relationship between individual leaf area (cm^2) and weight (g). Individual leaf values of thickness, leaf area, leaf
221 weight, and SLA were then averaged for the three leaves per plant.
222

223 **Leaf anatomy**
224 The anatomy of SD (High- and Low-A) and W *Silphium integrifolium* leaves of Experiment 1 was
225 compared by digitally measuring traits from scans of transverse sections. Three young, fully expanded leaves per
226 plant (10 reps/group High and Low A) were fixed in FAA (formaldehyde: 96% ethyl alcohol:glacial acetic
227 acid:water, 2:10:1:7, by vol.) and sent to the Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS to be
228 prepared, sectioned and stained.
229 Digital slides were stored at Case Center slide management system (3DHISTECH, the Digital Pathology
230 Company https://www.3dhistech.com/CaseCenter). InstantViewer, a multiplatform slide viewer application was
231 used to measure anatomical traits. Measured traits included: mesophyll density (number of cells per 20,000 μm^2),

232   mesophyll cell area (μm^2), mesophyll cell perimeter (μm), mesophyll air space (% of total mesophyll area), xylem
233   vessel area (μm^2), resin ducts area (% of cross section area), resin ducts number (number per unit area), palisade
234   mesophyll (%), spongy mesophyll (%) vascular tissue (%), and collenchyma (%)
235

236 Leaf chemistry

237   Leaves from SD and W genotypes in Experiment 1 were oven-dried at 40°C for 72 h and ground to fine
238   powder. Two g of dry, grounded leaf-biomass (each sample was a pool of 5 plants; 3 reps per SD and W genotype)
239   were extracted with methylene chloride (DCM; CH_2Cl_2; Sigma Aldrich, St. Louis, MO, USA) in a Soxhlet
240   apparatus, for 6 h. Extracts were evaporated with a rotary evaporator until dryness, and weighed. DCM extracts were
241   expressed as g of extract per g of dry leaf. Leaf nitrogen was measured using the standard Kjeldahl acid digestion
242   method (Scales and Harrison 1920).
243

244 Statistical analysis

245   Student´s t-tests were used to compare leaf morphological and anatomical traits, chemical composition and
246   gas exchange traits between W and SD plants and between high- and low- A groups within the SD plants. A two-
247   dimensional radar chart (Fig.1) was used to compare multiple traits of W and SD. The value of each trait is depicted
248   by the node on the axis. All the variables are connected to form a polygon. The Z-score for each trait is a number
249   that shows how many standard deviations the trait is above/below mean value (average of 120 SD plants; 20 W
250   plants). A two-way analysis of variance (plant group and year/stage) was carried out to determine differences in gas
251   exchange and anatomical traits. Interactions were analyzed. Data are expressed as mean ± standard error.
252   To investigate the relationships between gas exchange and anatomical traits we performed simple least
253   squares regressions analysis. To check for data normality, we used Shapiro−Wilk's test and Levene's test for
254   homogeneity of variance. Transformations (ln) were applied to water use efficiency (WUE, $\mu mol\ CO_2\ mmol\ H_2O^{-1}$),
255   whole leaf transpiration (E_{leaf}, $\mu mol\ leaf^{-1}\ s^{-1}$), Ci and AREA to satisfy the assumptions of ANOVA and regression
256   analysis. Statistical analyses were done using InfoStat Software, version 2014, Universidad Nacional de Córdoba,
257   Argentina.
258

259 RESULTS
260 Comparison of Semi-Domesticated vs Wild plants

261   Significant changes were found between morphological and physiological traits of leaves from W and SD
262   plants. The results are presented visually as radar charts to facilitate the comparison of groups along multiple
263   dimensions (Fig. 1). Leaves of SD plants had more than double the area ($T = 7.72$, $P < 0.01$), decreased thickness (T
264    $= -4.08$, $P < 0.01$), and increased weight ($T = 10.81$, $P < 0.01$), compared to those of W plants. SLA did not differ
265   between groups ($T = -1.63$, $P = 0.32$).
266   Stomatal conductance was higher in W than SD plants ($T = -6.79$, $P < 0.01$), with a concomitant difference
267   in transpiration; ($T = -11.5$, $P < 0.01$). Net CO_2 uptake (A_{area}, $\mu mol\ m^{-2}\ s^{-1}$) ($T = 3.67$, $P < 0.01$) was around 10%
268   higher in SD than W plants. To achieve this higher A_{area} [$\mu mol\ leaf^{-1}\ s^{-1}$] with lower g_s, SD plants required a lower
269   internal CO_2 concentration ($T = -3.43$, $P < 0.01$; Fig. 1). Because of the combined effect of higher A_{area} and larger
270   leaves, total Net CO_2 uptake per leaf of SD plants doubled that of W plants (A_{leaf}, $\mu mol\ leaf^{-1}\ s^{-1}$: $T = 5.72$, $P < 0.01$).
271   Whole leaf transpiration (E_{leaf}) was 25% higher in SD than in W plants ($T = 3.24$, $P < 0.01$).
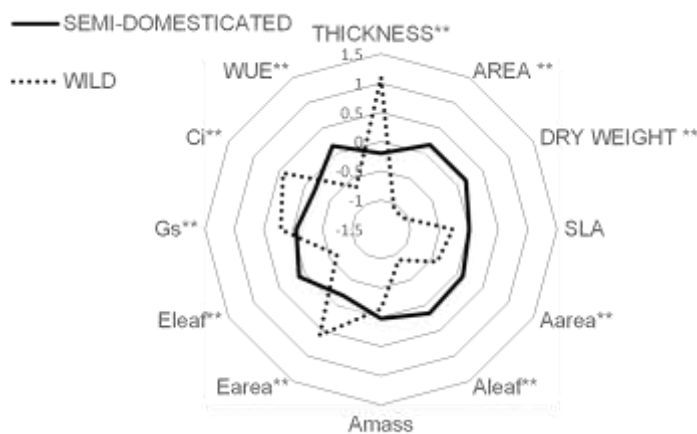272   Leaves of SD plants had higher nitrogen concentration (25.4 mg g^{-1} and 10.9 mg g^{-1} for SD and W,
273   respectively, $T = 3.41$, $P = 0.0022$) and total nitrogen content per leaf than those of wild plants (2.8 mg $leaf^{-1}$ and
274   16.7 mg $leaf^{-1}$ for W and SD, respectively). Resin content in the leaves was significantly higher in W than SD
275   accessions (8.8 ± 1.1 and 6.1 ± 0.8 for W and SD accessions, respectively; $T = 2.13$, $P = 0.04$).
276
277
278
279

Fig.1 Leaf morphological and gas exchange traits of wild and semi-domesticated (SD) accessions *Silphium integrifolium* grown in a common garden in Salina, Kansas (Experiment 1). The figure is based on Z-scores for the following traits: individual leaf area (Area), leaf dry weight (Dry Weight), leaf thickness (Thickness), specific leaf area (SLA), net CO_2 assimilation rate per area (A), transpiration (E), water use efficiency (WUE), stomatal conductance (Gs), internal CO_2 concentration (Ci), net CO_2 uptake of the whole leaf (Aleaf). Transpiration of a whole leaf (Eleaf), Net CO_2 uptake per unit leaf weight (Amass). Significant differences were found for all variables (** $P<0.01$), except for SLA and Amass. ($n = 120$ and 20, for SD and W accessions, respectively).

Extreme gas exchange phenotypes

Because a large range in Net CO_2 uptake and other gas exchange variables was found among the 120 individuals growing in the field, the 4 highest (High A, 8% of the population), and 4 lowest A plants (Low A, 8% of the population) were chosen for further measurements and analysis. These two groups of plants did not differ in seed-yield ($P = 0.91$; Yield $=57.6 \pm 27.3$ g seed per plant and 47.0 ± 11.3 g seed per plant, for High A and Low A plants, respectively; average of 2016 and 2017 harvests).

The differences in Net CO_2 uptake between the two groups was consistent between years and phenological stage of the plant. These differences between High A and Low A plants were also found in E, and g_s, and marginally in Ci, and nitrogen content (Table 1). No interaction between year/stage and photosynthesis group (High A and Low A) was found for any of the variables ($P > 0.29$ to 0.89 for all four variables).

Table 1 Instantaneous CO_2 uptake (A), transpiration rate (E), stomatal conductance (g_s), internal CO2 concentration (Ci), leaf N concentration, daily integrated net CO_2 uptake ($A_{integrated}$) and transpiration ($E_{integrated}$) for individual plants of High A and Low A. Measurements were taken on ten plants per group (and three leaves per plant) twice (bolting and flowering stages) in 2017 and twice in 2018. No interaction was found between photosynthesis group (High A and Low A) and year/season ($P>0.3$ for all variables), so the data were pooled for each group. Integrated values were measured in ten plants per group when plants were initiating bolting. Differences between years are not reported. No significant interaction was found between year and plant group.

Trait	High A	Low A	T	P
A [μmol m^{-2} s^{-1}]	25.6 ± 0.62	17.5 ± 0.67	1.19	<0.0001
E [mmol m^{-2} s^{-1}]	$0.011 \pm 8.1E^{0.4}$	$0.006 \pm 8.3 E^{0.4}$	1.23	0.0023
g_s [mmol m^{-2} s^{-1}]	0.46 ± 0.04	0.27 ± 0.03	0.91	<0.0001
Ci [ppm CO2]	270 ± 7.96	250 ± 8.56	3.6	0.105

Leaf N concentration [%d.w]	1.33 ± 0.13	1.68 ± 0.12	-1.98	0.069
$A_{integrated}$ [mol cm^{-2} day^{-1}]	0.87 ± 0.04	0.63 ± 0.06	11.58	< 0.01
$E_{integrated}$ [mol cm^{-2} day^{-1}]	236 ± 25	182 ± 13	6.41	< 0.05

311
312

313   Individuals of the High A and Low A groups also differed in the time-course of daily CO_2 uptake, but not
314 in E (Fig. 2). During the course of the day, A was higher for the High A group between sunrise and 13:00 h ($T = $
315 6.37, $P = 0.0003$ for $A_{integrated}$) when stomatal conductance was also higher ($T = -25.75$; $P < 0.0001$, for g_s averaged
316 from sunrise to 13:00), but not E ($T = 2.45$; $P < 0.13$). Neither A, E, g_s or Ci differed between High A and Low A
317 plants after 1 PM. Because of the higher instantaneous CO_2 uptake during the morning hours, plants in the High A
318 group had more total daily $A_{integrated}$ and total daily $E_{integrated}$ than those in the Low A group (Table 1).
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
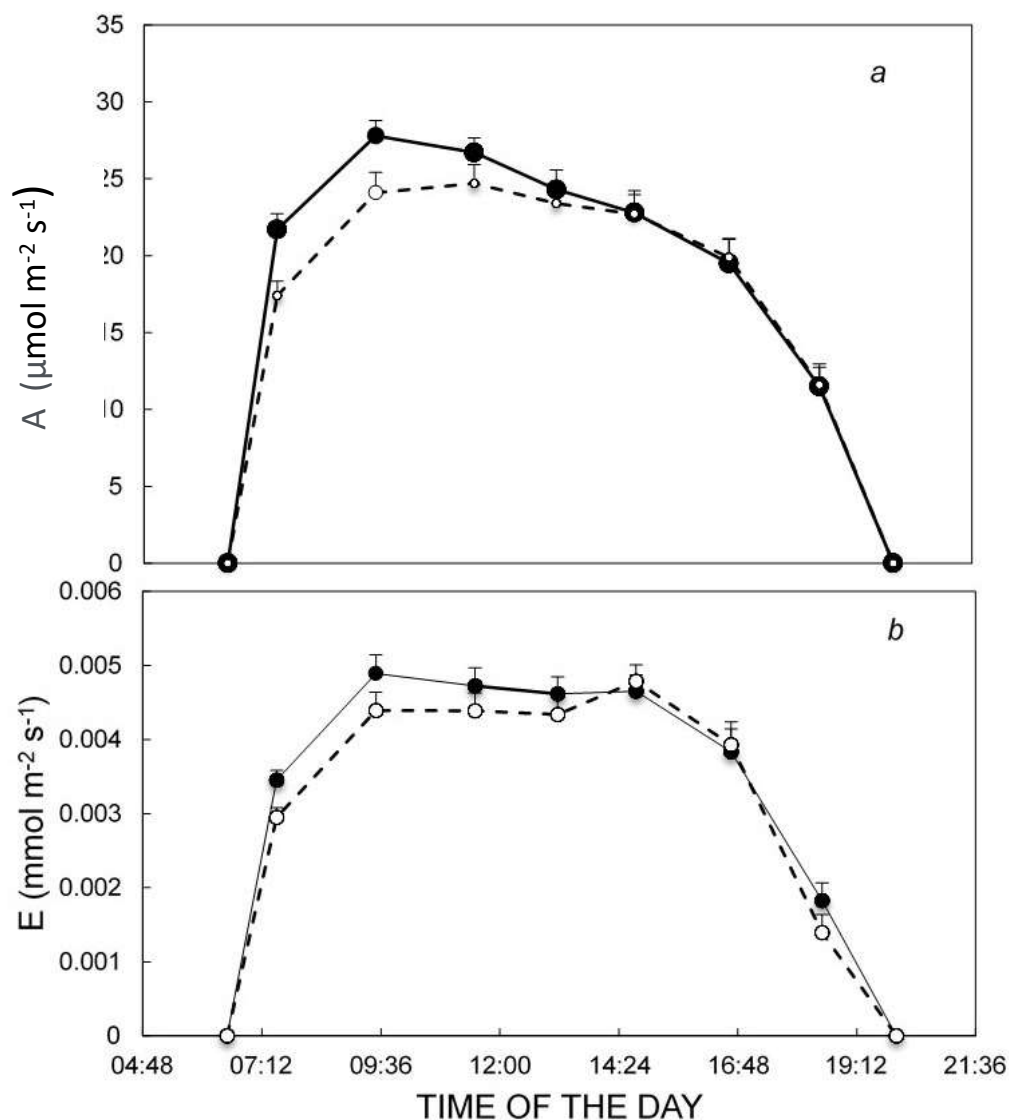
336
337
338
339 **Fig. 2** Daily course of Net CO_2 uptake **(a)** and transpiration **(b)** for High A and Low A individual
340 plants. Measurements were done on May 12, 2017, when plants started bolting. Black dots =High
341 A; White dots=Low A. Each point corresponds to 3 plants per group (8 leaves per plant). Values are means ±
342 standard error.

343

A/Ci curves: Biochemical traits limiting photosynthesis

345   High A and Low A plants grown in the field showed differences in the A/Ci response curves. High A plants
346 had higher Net CO_2 uptake over the entire range of external CO_2 availability, from 100 to 1200 ppm (Fig. 3a).
347   Further response curves were generated using clonal individuals of High A and Low A (Experiment 2; Fig.
348 3b). Visual inspection of these curves indicated triose phosphate utilization (TPU) limitation at high CO_2 levels.
349 $V_c$max, J, and TPU, and gm were significantly higher in High A than in Low A clones. There was a significant trend
350 of stomatal limitation (Ls, Table 2) with Low A plants being more limited than High A plants. Carbon compensation
351 point (Γ) did not differ between High A and Low A plants (Table 2). A linear significant relationship was found

352 between VcMax and Jmax for High A clones, although this relationship was not apparent for the Low A plants when
353 the two groups were analyzed separately (Fig. 4a and 4b).
354
355
356
357
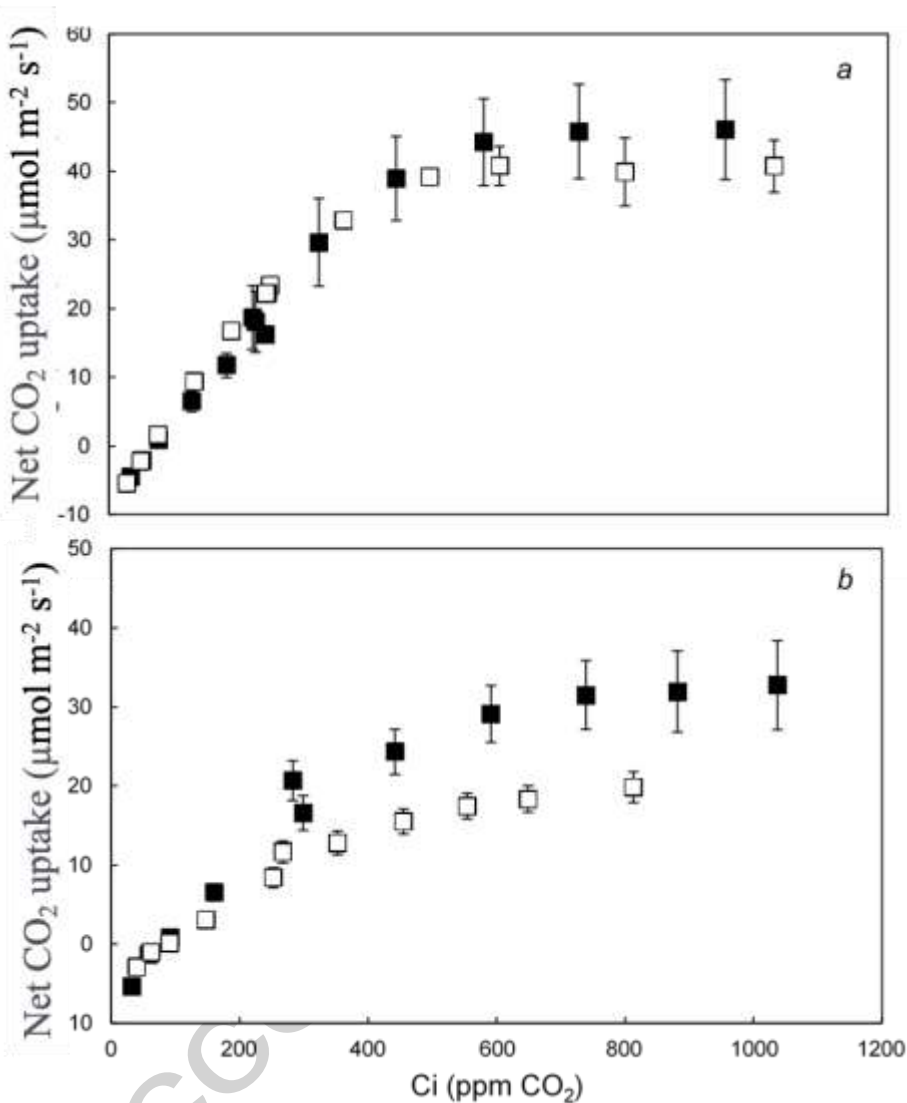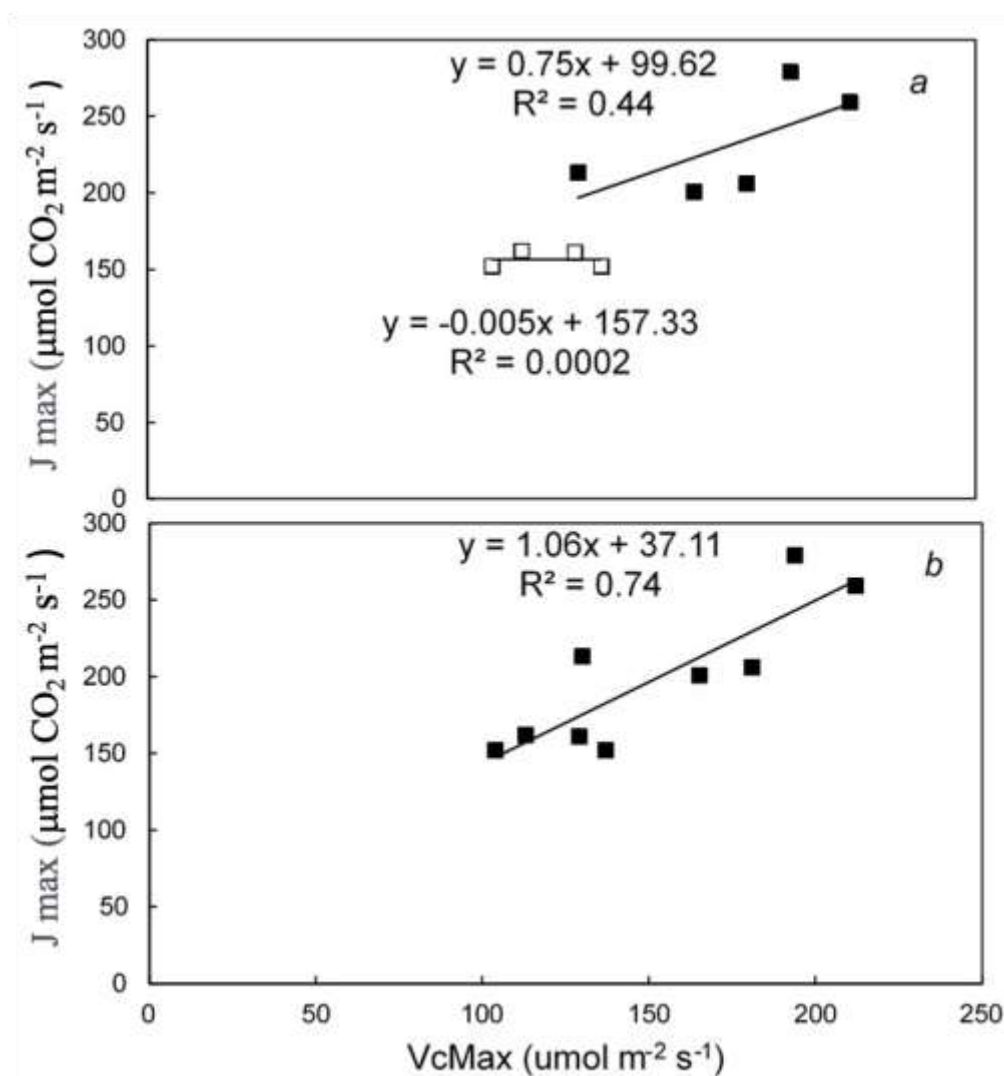

358
359
360
361
362 **Fig. 3** A/Ci curves for High A (black markers) and Low A (white markers) plants of *Silphium integrifolium* growing
363 in **a** in the field in Salina Kansas, on May 18, 2018 (*n*= 3 per group, Experiment 1) and **b** clones in the greenhouse
364 (*n* = 4 per group; Experiment 2).
365
366
367
368
369

370
371 **Table 2** Maximum RuBP carboxylation capacity ($V_c$max), electron transport rate (J), triose phosphate utilization
372 (TPU), day respiration (Rd), mesophyll conductance (gm), stomatal limitation (L_s) and carbon compensation point
373 (Γ), calculated for two groups of *Silphium integrifolium* plants: Cloned genotypes with stably High and Low
374 Assimilation rate (High A and Low A; Experiment 2). Individual potted clones of each genotype were treated as
375 subsamples. Means ± SE, *n*=4
376
377
378
379
380
381
382
383
384
385
386
387
388

Trait	High A clones	Low A clones	*T*	*P*
$V_c$max [µmol m^{-2} s^{-1} CO_2]	176.4 ± 13.9	120.7 ± 7.5	3.25	0.0140
J [µmol CO_2 m^{-2} s^{-1}]	231.6 ± 15.7	156.7 ± 2.7	4.69	0.0094
TPU [µmol CO_2 m^{-2} s^{-1}]	16.4 ± 1.2	10.4 ± 0.2	4.89	0.0081
Rd [µmol m^{-2} s^{-1}]	5.6 ± 0.4	4.0 ± 0.6	2.15	0.0686
gm [µmol m^{-2} s^{-1} Pa^{-1}]	3.6 ± 0.5	1.2 ± 0.2	4.09	0.0046
Ls [%]	25 ± 2	32 ± 4	-1.89	0.1007
Γ [µmol mol^{-1}]	65.6 ± 2.7	72.2 ± 4.3	-1.38	0.209

389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409

410
411
412

Fig. 4 a Relationship between the maximum rate of carboxylation (Vm_{max}) and electron transport (J_{max}) for High A (black markers) and Low A (white markers) clones of *Silphium integrifolium*, grown in a greenhouse (Experiment 2). Vc_{max} and J_{max} were estimated from A/Ci curves (Fig. 3b) following Sharkey et al. (2007) and using the calculator available at (https://onlinelibrary.wiley.com/page/journal/13653040/homepage/pce_calculator_2016.htm; Sharkey 2016) . **b** Relationship between the maximum rate of carboxylation (Vm_{max}) and electron transport (J_{max}) for High A and Low A clones of *Silphium* analyzed together

419

Leaf anatomy

421

Selection did not affect the relative allocation to leaf tissues, since the proportion of palisade and spongy mesophyll, collenchyma and vascular tissues was similar in W and SD, but it increased the epidermis thickness (Table 3) as well as the size of cells within the palisade mesophyll (Table 3; Fig. 5 b-d). Allocation to defense structures decreased: not only the number of resin ducts per area diminished but also the area of the ducts (Table 3 and Fig. 5a). Interestingly, the proportion of air space of the mesophyll was the only anatomical trait in which High- and Low-A plants significantly differ, and this trait was positively related to CO_2 uptake (Fig. 6a). The size of the

428 xylem vessels increased from wild to High A plants (Table 3). The leaves of SD plants had a thicker epidermis than
429 those of W plants; epidermis thickness was correlated with transpiration rate (E; Fig. 6b).

430
431

432 **Table 3** Comparison of anatomical traits of semi-domesticated (SD) and wild (W) accessions of *Silphium*
433 *integrifolium* growing at The Land Institute, Salina, KS. Mesophyll density was measured as the number of cells per
434 20,000 μm2; mesophyll air space was measured as the proportion of air in an area of 20,000μm^2; vascular tissues,
435 palisade and spongy mesophyll (%) was measured as the proportion of these tissues in the leaf cross section.
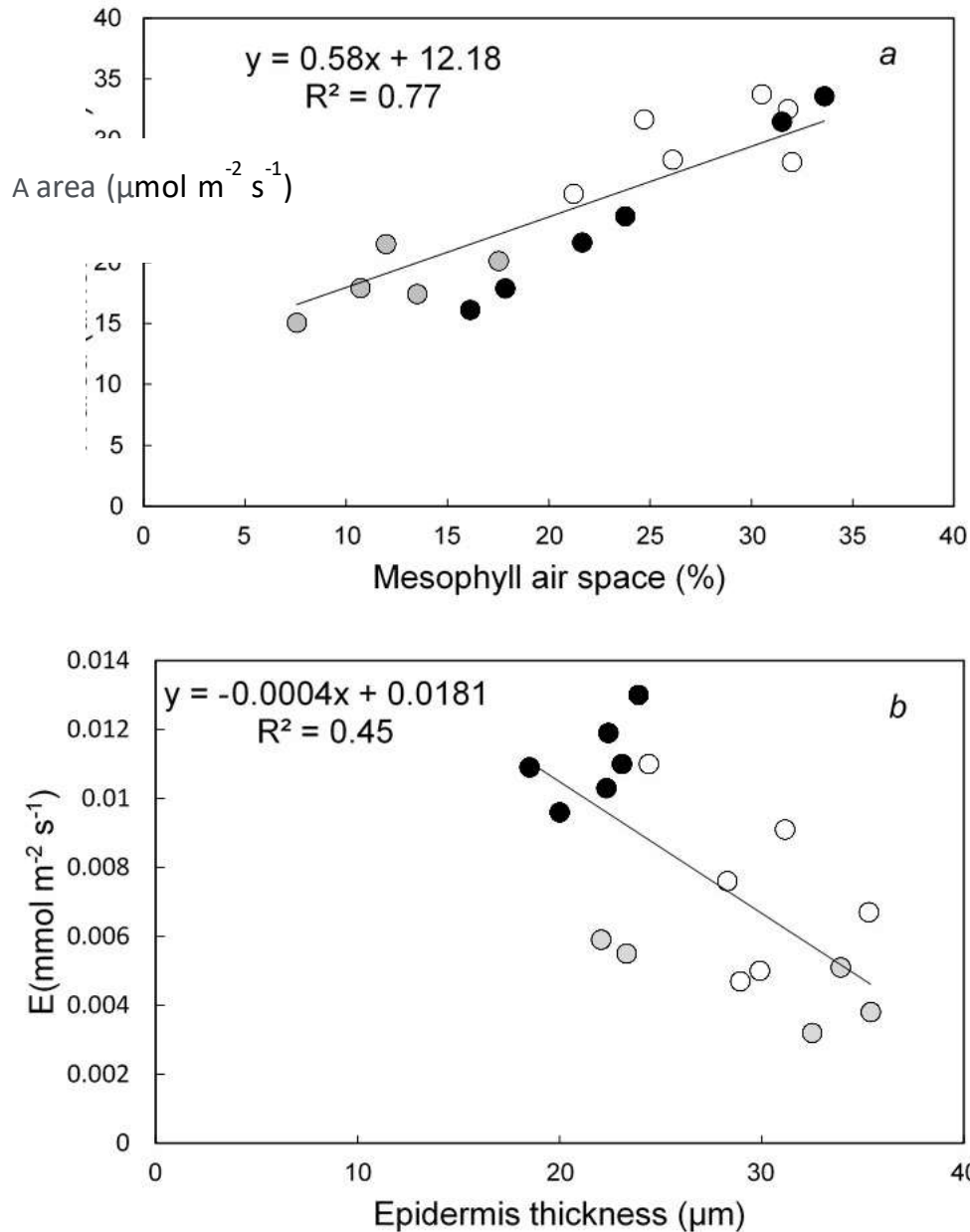436 Means ± SE, *n*=10

437
438
439
440
441
442
443
444

Anatomical traits	Semi-Domesticated (SD)		Wild	*F*	*df*	*P*
	SD High A	SD Low A				
Epidermis thickness [μm]	29.7 ± 1.8[b]	29.4 ± 2.8[b]	21.7 ± 0.8[a]	6.94	14	<0.01
Mesophyll density [cells per area]	16.7 ± 0.95[a]	17.0 ± 0.92[a]	21.4 ± 1.4[b]	5.67	14	0.02
Mesophyll cell area [μm^2]	1101.7 ± 93.0[b]	1073.2 ± 115.8[b]	493.5 ± 62.9[a]	14.92	17	<0.01
Mesophyll cell perimeter [μm]	153.9 ± 6.4[b]	152.0 ± 7.5[b]	92.1 ± 5.5[a]	30.65	17	<0.01
Mesophyll Air space [%]	27.7 ± 1.80[b]	12.2 ± 1.64[a]	24.1 ± 2.9[b]	12.07	14	<0.01
Xylem vessel area [μm^2]	1112.7 ± 40.5[b]	936.6 ± 130.0[ab]	722.5 ± 56.1[a]	6.65	14	<0.01
Resin ducts area [μm^2]	424.0 ± 149.4[a]	636.8 ± 165.7[ab]	1301.3 ± 280.3[b]	4.75	14	0.03
Resin ducts [n per area]	1.83 ± 0.65[a]	1.60 ± 0.5[a]	7.00 ± 0.9[b]	18.90	14	<0.01
Palisade mesophyll [%]	24.5 ± 3.47[a]	21.9 ± 2.6[a]	29.2 ± 1.5[a]	1.87	14	0.19
Spongy mesophyll [%]	32.1 ± 4.3[a]	37.4 ± 3.8[a]	34.8 ± 3.2[a]	0.47	14	0.63
Vascular tissues [%]	11.5 ± 1.0[a]	8.83 ± 0.5[a]	10.3 ± 1.8[a]	1.08	14	0.37
Collenchyma [%]	19.7 ± 3.2[a]	19.8 ± 3.8[a]	15.1 ± 1.8[a]	0.85	14	0.45

445
446
447
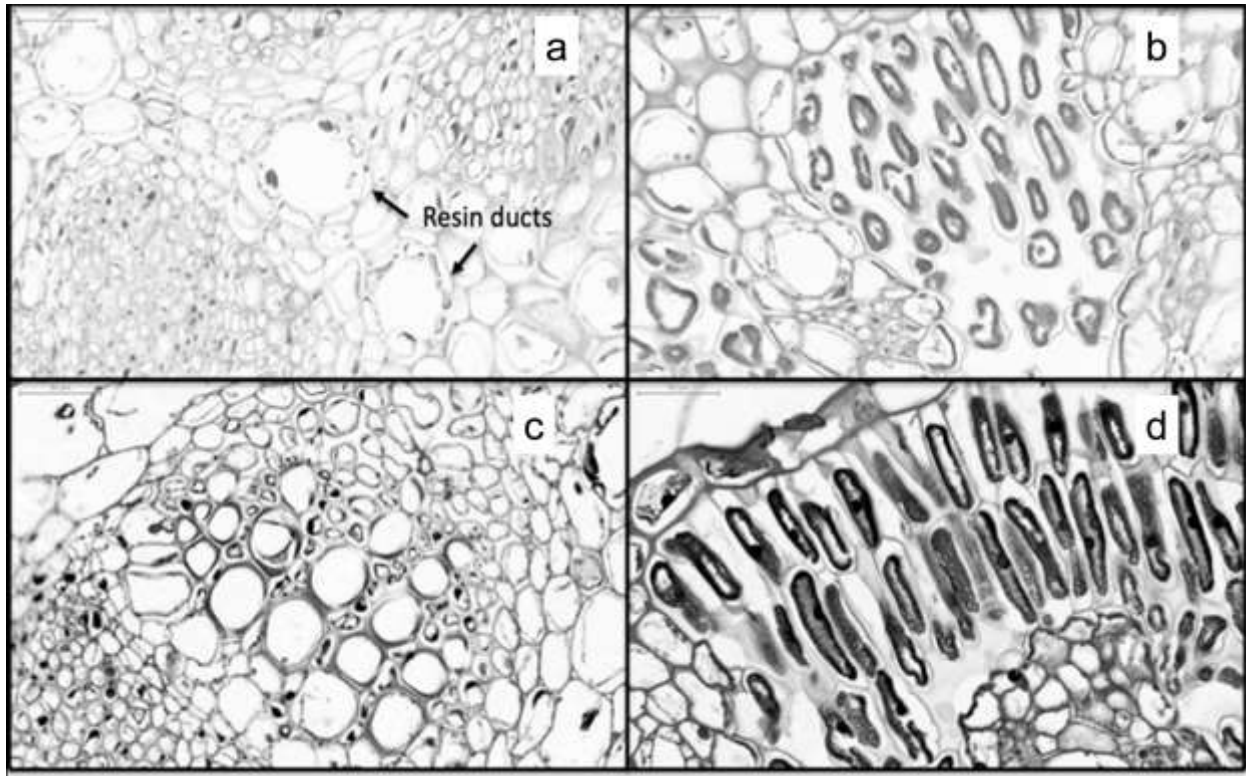
Fig. 5 Leaf cross-section of wild **(a-b)** and semi-domesticated plants **(c-d)** showing resin ducts **(a)**, palisade mesophyll **(b)** and metaxylem vessels **(c)**. Notice the increase in size of the mesophyll cells in high-A plants **(d)** compared to wild **(b)**. All pictures are taken at the same scale.

475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513

Fig. 6 Relationship between anatomical traits and gas exchange of leaves of *Silphium integrifolium* grown in the field in Salina, Kansas (Experiment 1). **a** Air space in the mesophyll and net CO_2 assimilation rate per unit area (A_{area}). **b** Epidermis thickness and transpiration rate (E). White markers are individual plants of high-A plants, grey markers correspond to Low A plants, and black markers to wild plants. *n*=6 for High A and Wild plants and 5 for Low A.

DISCUSSION

519
520   The domestication syndrome includes many traits that made plants easier to cultivate, harvest, and use by
521 humans. These changes occurred both at very early stages of unconscious plant domestication (Zohary 2004) and
522 later with conscious, directed selection and breeding focused on a few beneficial traits. Recently, the comparison of
523 wild and domesticated plants has been used to understand the domestication process, especially the way they acquire
524 carbon through photosynthesis, although very few studies report anatomical and physiological traits combined (Muir
525 et al. 2016, for an example in tomato).

526   Most the other studies that focus on the effects of domestication on plant function, use broad phylogenetic
527 scales and proxies such as SLA, to characterize leaf responses to domestication (Milla et al. 2014) or to evolution in
528 general (Donovan et al. 2011). Enhanced competitive ability (using SLA and leaf dry matter content as proxies) has
529 been found during early domestication but not necessarily in later stages (Milla et al. 2014), although there has been
530 a general lack of increase in area-based photosynthesis (Evans 1993; Milla and Matesanz 2017).

531   New domesticates, such as *Silphium integrifolium*, offer the rare chance to study early domestication using
532 conscious selection and breeding, combining processes that might have occurred sequentially in the development of
533 our current grain and oil-seed crops. Rapid evolution experiments that manipulate directed and natural selection (in
534 our case conscious and unconscious selection) have been proposed as a way to test the importance of diverse drivers
535 on domestication (Milla el at. 2015). Furthermore, working with wild perennial relatives can help understand early
536 domestication, since several of the ancestors of our current crops were perennial and lost perenniality during
537 domestication.

538   In our study, we found changes in leaf structure, anatomy and physiology after only five cycles of selection
539 (with seed-yield as the main selection criteria). Structural and functional traits were changed towards the acquisitive
540 side of the leaf economic spectrum (Wright et al. 2004). Leaves of SD plants were larger, heavier but thinner, larger
541 mesophyll cells, higher mesophyll conductance, had more nitrogen, higher net CO_2 uptake, higher whole-leaf
542 transpiration, less resin, and fewer resin ducts, than their W progenitors. Contrary to what was expected and
543 previously reported in several crops (Milla et al. 2013) leaves of SD plants had lower stomatal conductance and
544 internal CO_2 concentration, which allowed for higher water use efficiency. These reductions in g_s and Ci in SD
545 plants might be the consequence of selection occurring in a drier environment than that of the original mother wild
546 populations, which may have promoted higher water use efficiency. Still, because of the larger size, whole leaves
547 transpired 25% more water than those of the W plants.

548   The increase in leaf size and lack of change in SLA, is in line with previous findings that have shown there
549 is no consistent pattern of change in SLA under domestication (González et al. 1995; Wacker et al. 2002; Pujol et al.
550 2008) while it is clear that most anatomical and physiological differences observed between W and SD show
551 inadvertent selection towards the high resource-use end of the economic leaf spectrum (Wright et al. 2004). García-
552 Palacios et al. (2013) suggested that this lack of change in SLA during domestication is because the wild relatives of
553 domesticated plants were already high-resource strategists. Muir et al. (2016) offered a different explanation:
554 populations with similar SLA might nevertheless have different CO_2 diffusion rates due to leaf anatomical
555 differences. Thus, changes in CO_2 assimilation might be consistent with the predictions of the leaf economic
556 spectrum model, even though predictions about changes in SLA during domestication have failed. We found many
557 differences in leaf anatomy between leaves of W and SD plants that support Muir et al´s (2016) hypothesis.

558   The reduction in resin production and resin ducts is other significant change found that might be interpreted
559 as a shift from producing long-lasting, well-defended leaves towards producing leaves lower in construction costs
560 per unit leaf area. This shift would represent a more acquisitive strategy, enabling a plant to capture more radiation
561 by producing larger but cheaper leaves. Loss of defenses have been repeatedly found during domestication (Wink
562 1988; Turcotte et al. 2014). In fact, although the cause has yet to be established, some SD accessions of *Silphium*
563 with low resin and high nitrogen content were more susceptible to rust infection, and this infection promoted resin
564 production (Turner et al. 2018). The reduction in carbon based secondary metabolites appear to be more related to
565 changes in C:N ratios as found in other Asteraceae (Zavala and Ravetta 2001, 2002) than to a reduction in selective
566 pressure, since populations of both pests and pathogens appear to have increased for cultivated *Silphium* in these
567 large scale plots with no artificial pest control (Murrell, unpublished data).

568   In our study we found that selection for domestication traits provoked a coordinated change in anatomical
569 and physiological traits, as found recently in other perennial plants undergoing development as oilseed crops
570 (Gonzalez-Paleo and Ravetta 2015). To be able to increase CO_2 uptake with lower stomatal conductance, leaves of
571 SD plants have lower internal CO_2 concentration. Once the CO_2 reaches the substomatal cavity, it has to diffuse to
572 the inside of mesophyll cells and chloroplasts. Mesophyll conductance (gm) is used as an estimate of the internal
573 CO_2 diffusion. Because mesophyll conductance is inherently constrained by leaf structure (Niinemets et al. 2006),
574 the surface area of the mesophyll exposed to intercellular spaces should be maximized, to increase the area for CO_2
575 dissolution and the effective pathway for CO_2 diffusion and thereby, photosynthesis (Terashima et al. 2006, 2011).
576 SD plants had a less dense mesophyll, larger mesophyll cells and more air space (Table 3), which might be
577 conducive to an increased gm. We found a strong relationship between mesophyll air space and net CO_2 assimilation
578 (Fig. 6a), which supports the idea that gm changed during selection together with Net CO_2 uptake. Additionally,
579 xylem vessel area also increased with selection (Table 3) showing additional linkage between traits, since higher g_s
580 means the potential of a higher transpiration rate and the need of more reliable water supply to the stomata cavity.
581 Together with these changes we found a significant increase of the thickness of the epidermis brought about by
582 selection for seed-yield.
583   Artificial selection seems to have taken two different routes to support yield increase: one of high net CO_2
584 uptake (High A group) and one of low net CO_2 uptake (Low A group). These differences were consistent for 3 years
585 of field evaluation, and after that, when these plants were cloned for further study under controlled conditions.
586 Although we were not surprised to find many examples of weak or low-yielding individuals with reduced
587 photosynthetic capacity, the Low A group was vigorous and yielded as much as the High A group. High A and Low
588 A plants differed not only in instantaneous net CO_2 uptake, also had lower E, g_s and Ci. Over the course of the day,
589 High A plants had higher morning CO_2 uptake, and higher daily integrated uptake ($A_{integrated}$ and $E_{integrated}$). Both
590 groups had undergone similar changes in leaf anatomy that make them different to leaves of the wild genotype, but
591 differed strongly in mesophyll air space, in g_m, in g_s, and in stomatal limitation. The differences found between High
592 A and Low A plants opens the possibility to select plants with reproductive traits similar to those of acquisitive
593 annual crop yet with some leaf traits unchanged from the conservative wild ancestor (i.e., "low" A) resulting in a
594 more intermediate position on the leaf economic spectrum than is typical for domesticated crops
595   Phenotypic integration has been documented to occur in situations of environmental stress (Schlichting
596 1989). Hot summers, with the unpredictable drought conditions of central Kansas, could have provided the selection
597 pressure to develop the coordinated changes in leaf anatomy, morphology and function found in our study. High-A
598 plants, with a more acquisitive leaf strategy, should be able to achieve higher yields at the cost of higher
599 requirements of resources: higher nitrogen content and transpiration rate, mean more N and more water uptake from
600 the soil. Yield-stability might also be weakened by these changes in leaf structure and function (and growth) since
601 acquisitive plants tend to depend more on current resource availability than more conservative types (Low A group
602 with lower N content and transpiration rate). These findings point to the need to understand growth responses of
603 perennials to fluctuating resource availability, and the influence of carbon and nitrogen storage in maintaining
604 perenniality. Under water limiting conditions a High-A plant could potentially use carbohydrate reserves to buffer
605 lowered CO_2 uptake, while replenishing reserves during high water availability. On the other hand, a Low A plant
606 would maintain lower but steady rates of CO_2 uptake, with a more conservative use of water stored in the soil
607 throughout the season. These contrasting strategies need to be coupled with root structure and function; *Silphium* has
608 deep roots, but the extent of their functionality needs yet to be determined, as well as potential changes produced by
609 selection for yield. Preliminary data show root differences at least in seedlings (Gonzalez Paleo and Ravetta,
610 unpublished). Also, the use and recharge of carbohydrate reserves might impact perenniality, with reduced reserves
611 also reducing longevity as found in other perennial oil-seed crops (Gonzalez-Paleo and Ravetta 2015) and biomass
612 crops such as alfalfa (Castañeda et al. 2019).
613   Given the increased water use efficiency (WUE) enabled by the anatomical changes described above, it is
614 not surprising to find that some individuals perform high rates of A under different conditions, a strategy enabled by,
615 and requiring, high stomatal conductance. It is easy to imagine that selection for increased yield drove the changes

616 resulting in this acquisitive strategy, manifested by the High-A group. Finding an explanation for the Low A strategy
617 is not as straightforward as the acquisitive strategy.
618
619 **CONCLUSIONS**
620 Selection for increased yield during the early stages of domestication can result in rapid changes in leaf morphology,
621 anatomy and gas exchange that move plant strategy towards the acquisitive end of the leaf economic spectrum.
622 Starting from wild populations of *Silphium integrifolium*, 5 cycles of recurrent selection for seed yield components
623 and no conscious selection for vegetative traits produced plants with significantly larger, thinner leaves with bigger
624 palisade mesophyll cells and metaxylem vessels, presumably facilitating light capture and water supply.
625 An unexpected finding was that semi-domesticated plants showed contrasting physiological differences stable over
626 the course of plant development and two growing seasons. One group (High A) represents the expected transition to
627 a more acquisitive leaf strategy, while another group (Low A) had less net CO_2 uptake, but a more conservative
628 strategy of water use. Overall, these changes should have consequences for both provision (i.e., seed-yield) and
629 regulatory (i.e., sustainability) ecosystem services. The existence of different photosynthetic strategies opens the
630 possibility to select high yielding crops that do not increase drastically the use of resources. A crop intentionally
631 selected for conservative or intermediate leaf traits could provide economic and food benefits with fewer negative
632 effects on yield stability.
633
634 **AUTHORS´ CONTRIBUTION:** DAR, AEV and DVT conceived the ideas and designed methodology; DAR,
635 AEV, and LGP collected the data; DAR and AEV analyzed the data; DAR, AEV, LGP and DVT contributed to the
636 analysis and interpretation of data, and led the writing of the manuscript. All authors contributed critically to the
637 drafts and gave final approval for publication

638
639
640 **STATEMENTS AND DECLARATIONS**
641

645

646 **CONFLICTS OF INTERESTS:** Neither author has any conflict of interest.
647

648 **DATA AVAILABILITY STATEMENT:** Data supporting these results are available and can be provided by the
649 authors.
650
651

652 **REFERENCES**

653 Aber JD, Melillo JM, Mcclaugherty CA (1990) Predicting long-term patterns of mass loss, nitrogen dynamics, and
654 soil matter formation from initial fine litter chemistry in temperate forest ecosystems. Can J Bot 68: 2201-
655 2208
656 Aerts R, Chapin FS (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns.
657 Adv Ecol Res 30: 1-67. https://doi.org/10.1016/S0065-2504(08)60016-1
658 Austin AT, Yahdjian L, Stark J, Belnap MJ, Porporato AU, Norton U, et al. (2004) Water pulses and
659 biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141: 221-235
660 Brouillette LC, Mason CM, Shirk RY, Donovan LA (2014) Adaptive differentiation of traits related to resource use
661 in a desert annual along a resource gradient. New Phytol 201: 1316-1327
662 Castañeda VL, Marlon de la Peña A, Aranjuelo I, Gonzalez EM (2019) Functional analysis of the taproot and
663 fibrous roots of *Medicago truncatula*: Sucrose and proline catabolism primary response to water deficit.
664 Agric Water Manag 216: 473-483
665 Cates AM, Ruark MD, Hedtcke JL, Posner JL (2016) Long-term tillage, rotation and perennialization effects on
666 particulate and aggregate soil organic matter. Soil Tillage Res 155: 371-380
667 Coley PD, Bryant JP, Chapin FSIII (1985) Resource availability and plant antiherbivore defense. Science 230: 895-
668 899
669 Connor DJ, Loomis RS, Cassman KG (2011) Crop ecology. Productivity and management in agricultural systems.
670 Cambridge University Press. 576 pp, ISBN: 9780521744034
671 Cornell WK, Cornelissen HC (2013) A broader perspective on plant domestication and nutrient and carbon cycling.
672 New Phytol 198: 331-333
673 Cox TS, Bender MH, Picone C, Van Tassel DL, Holland JB, Brummer EC, Zoeller BE, Paterson AH, Jackson W
674 (2002) Breeding perennial grain crops. Crit Rev Plant Sci 21: 59-91
675 Cox TS, Glover JD, Van Tassel DL, Cox CM, DeHaan LR (2006) Prospects for developing perennial grain crops.
676 Bioscience 56: 649-659
677 Chacón-Fuentes M, Parra L, Rodriguez-Saona C, Seguel I, Ceballos R, Quiroz A (2015) Domestication in murtilla
678 (*Ugni molinae*) reduced defensive flavonol levels but increased resistance against a native herbivorous
679 insect. Environ Entomol 44: 627-637
680 Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plan functional traits and soil carbon sequestration in contrasting
681 biomes. Ecol Lett 11: 1-16
682 DeHaan LR, Van Tassel DL, Cox TS (2005) Perennial grain crops: a synthesis of ecology and plant breeding.
683 Renewable Agric Food Syst 20: 5-14
684 Denison RF (2012) Darwinian agriculture: How understanding evolution can improve agriculture. Princeton
685 University Press
686 Diamond J (2002) Evolution, consequences and future of plant and animal domestication. Nature 418: 700-707

687 Diaz S, Hodgson JG, Thompson K., et al (2004) The plant traits that drive ecosystems: Evidence from three
688   continents. J Veg Sci 15: 295-304

689 Diaz S, Kattge JM, Kleyer C, Wirth IC, Prentice E, Garnier G, Bönisch M, Westoby M (2016) The global spectrum
690   of plant form and function. Nature 529: 167-171

691 Donovan L, Maherali H, Caruso C, Huber H, Kroon H (2011) The evolution of the world-wide leaf economic
692   spectrum. Trends Ecol Evol 26: 86-95

693 Evans LT (1993) Crop evolution, adaptation and yield. Cambridge University Press, Cambridge, UK

694 Farquhar GD, Von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in
695   leaves of C3 species. Planta 149: 78-90

696 Flexas J (2016) Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: Why so
697   much little success?. Plant Sci 251: 155-161

698 García-Palacios P, Milla R, Delgado-Baquerizo R, Martin-Robles M, Alvaro-Sanchez N, Wall DH (2013) Side-
699   effects of plant domestication: ecosystem impacts of change litter quality. New Phytol 198: 504-513

700 Gifford RM, Evans LT (1981) Photosynthesis, carbon partitioning and yield. Annu Rev Plant Physiol 32: 485-509

701 Gepts, P (2004) Crop domestication as a long-term selection experiment. Plant Breed Rev 24 (Part 2): 1-44 (Janick
702   J, ed). © 2004 John Wiley & Sons, Inc., ISBN 0-471-46892-4

703 Glover JD, Reganold JP (2010) Perennial grains food security for the future. Issues Sci Technol 26. 2

704 Gols R, Bukovinszky T, Van Dam NM, Dicke M, Bullock JM, Harvey JA (2008) Performance of generalist and
705   specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. J Chem
706   Ecol 34: 132-143

707 González A, Lynch J, Tohme J, Beebe S, Macchiavelli R (1995) Characters related to leaf photosynthesis in wild
708   populations and landraces of common bean. Crop Sci 35: 1468-1476

709 Gonzalez-Paleo L, Ravetta DA (2011) Relationships between reproductive output, morpho-physiological traits and
710   life span in *Lesquerella* (Brassicaceae). Ind Crops Prod 34: 1386-1392

711 Gonzalez-Paleo L, Ravetta DA (2015) Comparison of growth and carbon use strategy of annual and perennial
712   species of desert forbs. Flora 21:1-9

713 Gonzalez-Paleo L, Vilela AE, Ravetta DA (2016) Back to perennials: Does selection enhance tradeoffs between
714   yield and longevity?. Ind Crops Prod 91:272-278

715 Gonzalez-Paleo L, Pastor-Pastor A, Rajnoch G, Ravetta DA (2019) Mechanisms of nitrogen conservation at the leaf-
716   level in annual and perennial desert forbs: Implications for perennial crops domestication. Flora 252: 62-68

717 Gonzalez-Paleo L, Ravetta DA, Van Tassel D (2022) From leaf traits to agroecosystem functioning: effects of
718   changing resource use strategy during *Silphium* domestication on litter quality and decomposition rate.
719   Plant Soil 471(1-2):1-13

720 Harlan RS (1973) Comparative evolution of cereals. Evolution 27: 311-325

721 Jaikumar NS, Snapp SS, Sharkey TD (2013) Life history and resource acquisition: photosynthetic traits in selected
722   accessions of three perennial cereal species compared with annual wheat and rye. Am J Bot 100: 2468-2477

723 Kantar M, Tyl C, Dorn K, Zhang X, Jungers J, Kaser JM, et al (2016) Perennial grain and oilseed crops. Annu Rev
724   Plant Biol 67: 703-729

725 Long SP, Bernachi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations in
726   photosynthesis?. Procedures and sources of error. J Exp Bot 54: 2393-2401

727 Long SP, Zhu XG, Naidu SL, Ort DR (2006) Can improvement in photosynthesis increase crop yields?. Plant Cell
728   Environ 29: 315-330

729 Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: An historical review and
730   quantitative analysis of 203 global food crops. New Phytol 196: 29-48

731 Milla R, Vico N, Martin-Robles N (2013) Shifts in stomatal traits following the domestication of plant species. J
732   Exp Bot 64: 3137-3146

733 Milla R, Morente-López J, Alonso-Rodrigo JM, Martín-Robles N, Chapin FS (2014) Shifts and disruptions in
734   resource-use trait syndromes during the evolution of herbaceous crops. Proc R Soc Lond B Biol Sci
735   281(1793):20141429. doi: 10.1098/rspb.2014.1429

736   Milla R, Osborne C, Turcotte M, Violle C (2015) Plant domestication through an ecological lens. Trends Ecol Evol
737   30: 463-469

738   Milla R, Matesanz S (2017) Growing larger with domestication: a matter of physiology, morphology or allocation.
739   Plant Biol 19: 475-483

740   Mondolot L, Marlas A, Barbeau D, Gargadennec A, Pujol B, McKey D (2008) Domestication and defense: Foliar
741   tannins and C/N ratios in cassava and a close wild relative. Acta Oecol 34: 147-154

742   Muir CD, Conesa M, Roldán EJ, Molins A, Galmés J (2016) Weak coordination between leaf structure and function
743   among closely related tomato species. New Phytol 213: 1642-1653

744   Niinemets U, Cescatti A, Rodeghiero M, Tosens T (2006) Complex adjustments of photosynthetic capacity and
745   internal mesophyll conductance to current and previous light availabilities and leaf age in Mediterranean
746   evergreen species *Quercus ilex*. Plant Cell Environ 29: 1159-1178

747   Pastor-Pastor A, Vilela AE, González-Paleo L (2018a) The root of the problem of perennials domestication: Is
748   selection for yield changing key root system traits required for ecological sustainability?. Plant Soil 435:
749   161-174

750   Pastor-Pastor A, Vilela AE, González-Paleo L (2018b) Tradeoffs between productivity and nitrogen conservation in
751   wild and domesticated plants of the perennial crop *Physaria* (Brassicaceae). Ann App Biol 173: 121-132

752   Pimentel D, Cerasale D, Stanley RC, Perlman R, Newman EM, Brent LC, et al (2012) Annual vs perennial grain
753   production. Agric Ecosyst Environ 161: 1-9

754   Poorter H, De Jong R (1999) A comparison of specific leaf area, chemical composition and leaf construction costs of
755   field plants from 15 habitats differing in productivity. New Phytol 143:163-176

756   Poorter H, Garnier E (1999) Ecological significance of inherent variation in relative growth rate and its components.
757   In: Pugnaire FI, Valladares F (eds) Handbook of plant functional ecology. Marcel Dekker, New York, pp
758   81-120

759   Pujol B, Salager JL, Beltran M, Bousquet S, McKey D (2008) Photosynthesis and leaf structure in domesticated
760   *Cassava* (Euphorbiaceae) and a close wild relative: have leaf photosynthetic parameters evolved under
761   domestication?. Biotropica 40: 305-312

762   Ravetta DA, Soriano A (1998) Alternatives for the development of new industrial crops for Patagonia. Ecologia
763   Austral 8: 297-307

764   Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc
765   Natl Acad Sci USA 94: 13730-13734

766   Reich PB (1998) Variation among plant species in leaf turnover rates and associated traits: implications for growth
767   at all life stages. In: Lambers H, Poorter H, Van Vuuren MM (eds) Inherent variation in plant growth.
768   Physiological mechanisms and ecological consequences. Leiden, Netherlands: Backhuys Publishers, pp
769   467-487

770   Scales FM, Harrison AP (1920) Boric acid modification of the Kjeldahl method for crop and soil analysis. J Ind Eng
771   Chem 12: 350-352

772   Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves
773   for C3 leaves. Plant Cell Environ 30:1035-1040

774   Sharkey TD (2016) What gas exchange data can tell us about photosynthesis?. Plant Cell Environ 39: 1161-1163

775   Schlichting CD (1889) Phenotypic interaction and environmental change. Bioscience 39: 460-464

776   Terashima I, Hanba YT, Tazoe Y, Vyas P, Yano S (2006) Irradiance and phenotype: comparative eco-development
777   of sun and shade leaves in relation to photosynthetic CO_2 diffusion. J Exp Bot 57: 343-354

778   Terashima I, Hanba YT, Tholen D, Niinemets Ü (2011) Leaf functional anatomy in relation to photosynthesis. Plant
779   Physiol 155: 108-116

780   Tholen D, Boom C, Zhu XG (2012) Opinion: Prospects for improving photosynthesis by altering leaf anatomy.
781   Plant Sci 197: 92-101

782   Turcotte M, Nash M, Turley E, Johnson MTJ (2014) The impact of domestication on resistance to two generalist
783   herbivores across 29 independent domestication events. New Phytol 204: 671-681

784 Turner MK, Ravetta DA, Van Tassel DL (2018) Effect of *Puccinia silphii* on yield components and leaf physiology
785   in *Silphium integrifolium*: Lessons for the domestication of a perennial oilseed crop. Sustainability 10: 1-12
786 Van Tassel DL, DeHaan LR, Cox TS (2010) Missing domesticated plant forms: Can artificial selection fill the gap?.
787   Evol App 3: 434-452
788 Van Tassel DL, Asselin SR, Cox SA, Sideli G, Cattani DJ (2014) Evaluating perennial candidates for domestica-
789   tion: Lessons from wild sunflower relatives. In: Batello C, Wade L, Cox S, Pogna N, Bozzini A, Choptiany
790   J (eds) Perennial crops for food security. Proceedings of the FAO Expert Workshop, FAO, Rome. pp 112–
791   140
792 Vico G, Manzoni S, Nkurunziza L, Murphy K, Weih M (2016) Trade-offs between seed output and life span – a
793   quantitative comparison of traits between annual and perennial congeneric species. New Phytol 209: 104-
794   114. https://doi.org/10.1111/nph.13574

795 Vilela AE, González-Paleo L (2015) Changes in resource-use strategy and phenotypic plasticity associated with
796   selection for yield in wild species native to arid environments. J Arid Environ 113: 51-58
797 Vilela AE, González-Paleo L, Turner K, Peterson K, Ravetta DA, Crews TE, Van Tassel DL (2018) Progress and
798   bottlenecks in the early domestication of the perennial oilseed *Silphium integrifolium*, a sunflower
799   substitute. Sustainability 10: 638
800 Wacker L, Jacomet S, Korner C (2002) Trends in biomass fractionation in wheat and barley from wild ancestors to
801   modern cultivars. Plant Biol 4: 258-265
802 Westoby M, Falster DS, Moles AT et al (2002) Plant ecological strategies: Some leading dimensions of variation
803   between species. Annu Rev Ecol Evol Syst 33: 125-159
804 Whitehead SR, Turcotte MM, Poveda K (2017) Domestication impacts on plant–herbivore interactions: A meta-
805   analysis. Phil Trans Royal Soc B: Biol Sci 372: article ID 20160034. doi: 10.1098/rstb.2016.0034.
806 Wink M (1998) Plant breeding: importance of plant secondary metabolites for protection against pathogens and
807   herbivores. Theor Appl Genet 75: 225-233
808 Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. Nature 428: 821-827
809 Zavala J, Ravetta DA (2001) The effect of irrigation regime on biomass and resin production in *Grindelia chiloensis*.
810   Field Crops Res 69: 227-236
811 Zavala J, Ravetta DA (2002) The effect of solar U-V radiation on biomass partition, growth and resin production on
812   *Grindelia chiloensis*. Plant Ecol 16: 185-191
813 Zohary D (2004) Unconscious selection and the evolution of domesticated plants. Econ Bot 58: 5-10

814
815
816 **FIGURE LEGENDS**
817
818 **Fig. 1** Leaf morphological and gas exchange traits of wild and semi-domesticated (SD) accessions *Silphium*
819 *integrifolium* grown in a common garden in Salina, Kansas (Experiment 1). The figure is based on Z-scores for the
820 following traits: individual leaf area (Area), leaf dry weight (Dry Weight), leaf thickness (Thickness), specific leaf
821 area (SLA), net CO_2 assimilation rate per area (A), transpiration (E), water use efficiency (WUE), stomatal
822 conductance (Gs), internal CO_2 concentration (Ci), net CO_2 uptake of the whole leaf (Aleaf). Transpiration of a
823 whole leaf (Eleaf), Net CO_2 uptake per unit leaf weight (Amass). Significant differences were found for all
824 variables (** $P<0.01$), except for SLA and Amass. ($n = 120$ and 20, for SD and wild accessions, respectively).
825
826
827 **Fig. 2** Daily course of Net CO_2 uptake **(a)** and transpiration **(b)** for High A and Low A individual
828 plants. Measurements were done on May 12, 2017, when plants started bolting. Black dots = High
829 A; White dots = Low A. Each point corresponds to 3 plants per group (8 leaves per plant). Values are means ±
830 standard error
831
832
833 **Fig. 3** A/Ci curves for High A (black markers) and Low A (white markers) plants of *Silphium integrifolium* growing
834 in **a** in the field in Salina Kansas, on May 18, 2018 ($n= 3$ per group, Experiment 1) and **b** clones in the greenhouse
835 ($n = 4$ per group; Experiment 2)
836
837
838 **Fig. 4 a** Relationship between the maximum rate of carboxylation (Vm$_{max}$) and electron transport (J$_{max}$) for High A
839 (black markers) and Low A (white markers) clones of *Silphium integrifolium*, grown in a greenhouse (Experiment
840 2). Vc$_{max}$ and J$_{max}$ were estimated from A/Ci curves (Fig. 3b) following Sharkey et al. (2007) and using the
841 calculator available at (https://onlinelibrary.wiley.com/page/journal/13653040/homepage/pce_calculator_2016.htm;
842 Sharkey 2016). **b** Relationship between the maximum rate of carboxylation (Vm$_{max}$) and electron transport (J$_{max}$) for
843 High A and Low A clones of *Silphium* analyzed together
844
845 **Fig. 5** Leaf cross-section of wild **(a-b)** and semi-domesticated plants **(c-d)** showing resin ducts **(a)**, palisade
846 mesophyll **(b)** and metaxylem vessels **(c)**. Notice the increase in size of the mesophyll cells in high-A plants **(d)**
847 compared to wild **(b)**. All pictures are taken at the same scale
848
849 **Fig. 6** Relationship between anatomical traits and gas exchange of leaves of *Silphium integrifolium* grown in the
850 field in Salina, Kansas (Experiment 1). **a** Air space in the mesophyll and net CO_2 assimilation rate per unit area
851 (A$_{area}$). **b** Epidermis thickness and transpiration rate (E). White markers are individual plants of high-A plants, grey
852 markers correspond to Low A plants, and black markers to wild plants. $n=6$ for High A and W plants up and 5 for
853 Low

854

855