09

EVALUATING PERENNIAL CANDIDATES FOR DOMESTICATION:
LESSONS FROM WILD SUNFLOWER RELATIVES

David L. Van Tassel[1]*, Sean R. Asselin[2], Sheila A. Cox[1], Gina Sideli[3], Douglas J. Cattani[2]

1   The Land Institute, Salina, Kansas, USA

2   University of Manitoba, Department of Plant Science, Winnipeg, Manitoba, Canada.

3   California State University, Biological Sciences Department, Chico, California, USA

*   Corresponding Author: The Land Institute, 2440 E. Water Well Rd., Salina, Kansas, 67401, USA.
    Phone: (+1) 785 823 5376; Email: vantassel@landinstitute.org

ABSTRACT

Recurrent selection for increased seed production has been successful in two wild perennial species related to sunflower, *Helianthus maximiliani* and *Silphium integrifolium*. A third species, *Helianthus pauciflorus* has highly heritable variation for seed yield and should respond to selection. Each wild species has a different set of traits that limit its usefulness as a grain crop and each has unique adaptations beyond the perennial habit that could enhance its value or accelerate its domestication. For example, *S. integrifolium* is deeply rooted and relatively unaffected by short term droughts, while *H. pauciflorus* is easily asexually propagated by tubers making clonal cultivars feasible.

More generally, we ask which traits make a wild perennial more genetically and agronomically tractable and which complex but ecologically important traits might only be brought to the agro-ecosystem through domestication? Any perennial grain reduces the need for tillage, but perennials are not equally drought tolerant, competitive with weeds or carbon sequestering. We suggest, from both experience and deduction, that seed yield and the classic domestication traits such as shattering may often be less important in selecting perennial species for domestication than genetically complex traits such as growth rate, phenology or branching pattern.

Keywords: perennial grains, oil crops, oilseeds, domestication, landscape genetics, crop wild relatives, functional traits, *Silphium, Helianthus, Linum*

INTRODUCTION

Perennial grains, including perennial cereals, pulses and oilseeds, are being investigated or developed at several institutions as a long-term solution to the ongoing problem of soil erosion and degradation associated with annual grain cropping systems. The rationale for increasing the proportion of agricultural land planted to perennial crops has been more fully articulated by Glover *et al.* 2010; while the potential physiological and genetic obstacles to combining high grain yield and multi-year lifespan—and suggestions for how these obstacles can be overcome-have also been explored elsewhere (DeHaan *et al.* 2007; Van Tassel *et al.* 2010). In this volume given this rationale we assume that high-yielding perennial oilseeds are both beneficial and possible to develop. We provide a rationale for investing in new oilseed crops in addition to the more obvious cereals and for choosing domestication in these cases instead of using wide hybridization to "perennialize" an existing crop. Next we survey the native North American species being domesticated as perennial oilseeds. We report progress and observations with these species, and describe our plans for continued domestication. Finally, we compare the candidates and look for principles that could help future domesticators identify candidates likely to respond rapidly to domesticating selection from among the many seed-bearing wild species.

I. Rationale

Perennial oilseed grains will contribute to long-term food security and renewable energy.
Oil crops contribute to food security in every region because they are less expensive than animal fats and dietary lipids supply both calories and the essential fatty acids alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid) that humans cannot synthesize. Furthermore, some fat in the diet greatly improves the bioavailability of at least one

vitamin precursor, B-carotene (van het Hof *et al.* 2000), and could increase the uptake of other fat-soluble nutrients such as lycopene, lutein, Vitamins K and D (Brown *et al.* 2004).

Oilseed crops play an important role in the diversity of cropping systems, providing functional diversity to compliment grain and legume rotations. Many oilseeds are duel or multi-purpose crops, filling multiple end uses (Vollamann and Rajcan, 2010). For instance crops such as sunflower, soybean and canola provide human nutrients, protein-rich meal for animal consumption, and the raw feedstock for biofuels (Agarwal, 2007) and bioplastics and other industrial products (Xia and Larock, 2010) which can aid in reducing the dependency of petroleum based fossil fuels.

Perhaps the most compelling argument in favour of perennial oilseed research is simply that oil crops currently occupy 281 million hectares (FAOstat, 2012 data). This is about 26 percent of the world's acreage devoted to staple crops (cereals, pulses, oilcrops and roots/tubers) and about 18 percent of all land classified as arable or under permanent crops (FAOstat, 2011 data). For whatever reasons, humanity has chosen to devote a large proportion of the planet's land area to oil crops and providing more perennial oil crop options could reduce the ecological disservices associated with annual oil crops. Encouragingly, 41 percent of all vegetable oil already comes from perennials such as olive, oil palm, and coconut (FAOstat, 2012 data).

However, these perennial oil crops are all tropical or subtropical tree-like species and virtually all the oil crops in temperate regions are annuals such as soybean, sunflower, flaxseed and canola. Furthermore, tree-like crops are slow to establish and expensive to harvest and immobilize moisture and nutrients from the environment for extended periods. Perennial oilseed forbs could provide additional options even in the tropics, supplying some of the ecosystem services of the tree crops, as well as new services, such as fodder, and new harvesting and management (mowing, grazing and burning) options.

In both tropical and temperate regions, the development of perennial oilseeds, particularly those with diverse end-uses and capable of producing on marginal lands where annual cropping is at a high risk of failure, has the potential to increase the productivity of global agricultural systems.

Domestication of perennial oilseeds, perennialization of domestic oilseeds and intermediate approaches. There are currently four main routes to combining the yield of an annual crop with the longevity of a wild perennial. The first three require wide hybridization between an annual species and a perennial species. Under the "triticale option", a full or partial amphiploids with whole chromosomes of both species is created. If the chromosome constitution remains stable in successive generations, a new species has been created. Option 2 utilizes introgression breeding to move "domestication genes" from the cultigen into the wild perennial background. Option 3 is similar, but aims to move "perennialism genes" from the wild species into the genetic background of the cultivated species. The fourth option—domestication—attempts to find rare alleles in the primary gene pool of a wild perennial that confer favourable "domestication syndrome" traits such as reduced shattering, increased threshability, etc.

All of these routes are being attempted with members of the sunflower family. Researchers at The University of Minnesota (Hulke and Wyse, 2008; Kantar *et al.* 2010; Kantar *et al.* 2014) made wide hybrids between *Helianthus tuberosus* and the crop sunflower (*Helianthus annuus*) followed by using the annual (diploid or tetraploid lines) as the recurrent parent. Two of us (Van Tassel and Cox) have attempted to select for stable amphiploids from among the progeny of intermated wide hybrids between either *Helianthus tuberosus* or *H. pauciflorus* (perennials) and the crop sunflower. While the details of these programs are beyond the scope of this report, the summary is that stable amphiploids are very rare and exhibit low fertility. Even if found, these lines are unlikely to contain the allelic diversity needed for long-term crop improvement and cultivar development. Additional amphiploids of the same karyotype will have to be developed for that. Similarly, crossing hybrids with either the annual or perennial parent often appears to result in the elimination of many or all of the chromosomes of one species or the other.

The situation appears to be very similar to that encountered when trying to transfer genes from *Tripsacum* to *Zea*. Harlan and DeWet (1977) wrote:

The cytogenetic interactions in maize x *Tripsacum* hybrid derivatives can be remarkably complex. The number of viable, somewhat female fertile chromosome combinations is astonishing. Fifty-four different combinations are listed in this paper, and others are certainly possible. In successive backcrosses to maize, chromosome numbers scale both up and down with ease, sometimes stabilizing for a few generations via apomixis, but in most cases finally generate balanced maize genomes and emerge as recovered maize. The maize, however, can produce strange and unusual phenotypes as a result of genetic transfers from *Tripsacum*. Highly tripsacoid maize lines with 2n = 20 chromosomes were recovered in pathways where the maize genome had first contaminated the *Tripsacum* genome in early backcross generations. In other pathways interaction of the two genomes is rare.

Cox is currently attempting to develop "annuus-oid" *H. pauciflorus* by making numerous F1 hybrids between *H. pauciflorus* and *H. annuus*, using the perennial as the female and then "backcrossing" the F1s to the perennial until a fertile, balanced *H. pauciflorus* genome is recovered. Her aim is to introgress a few "domestication genes" into *H. pauciflorus*, and her hypothesis is that even a few alien alleles increasing seed or head size could greatly accelerate the domestication of *H. pauciflorus*. It seems to these authors that introgressing the perennial habit into the annual background is a more daunting task because several to many alien genes are likely to be required simultaneously in order to produce a perennial and winter-hardy phenotype.

Domestication: slow but steady. In contrast with wide hybridization, fertile parents in a domestication population are intermated and fertile offspring are produced which can be easily intermated, crossed with new, wild individuals, or backcrossed to their parents. The "astonishing" number of cytotypes and dramatic "strange and unusual" phenotypes that Harlan and DeWet (1977) saw with the derivatives of interspecific hybrids and that Van Tassel and Cox have seen

with interspecific Helianthus are not generated by this approach. There is no possibility of making a single cross that fortuitously combines all the desirable traits of the domestic species and the longevity and hardiness of the perennial. But, on the other hand, serious problems with sterility, chromosome number fluctuation and development of small populations with strong barriers to gene flow with other sources of genetic variation are unlikely to be encountered. Domestication is a slow and steady strategy unlikely to produce dramatic "early wins" but also unlikely to fail if given enough time. In experimental populations, recurrent phenotypic selection on individuals has repeatedly resulted in the evolution of novel phenotypes (e.g. Ratcliff *et al.* 2012; reviewed by Travisano and Shaw, 2013).

Recombination load is the reduction of fitness caused by breaking favourable genetic associations that have been generated by selection (Otto and Lenormand, 2002). Presumably the compliment of chromosomes in a genome represents one of the strongest forms of genetic association. The addition of entire or large pieces of alien chromosomes is almost certain to disrupt the previous genetic associations. Beyond disrupting the process of meiosis itself, wide crossing recombines whole developmental programs sometimes leading to misshapen, fragile or non-functional organs (Siikamaki, 1999). Any adaptations involving whole plant architecture and/or finely tuned phenology are likely to be lost following wide hybridization. On the other hand, the crop field may represent such a novel environment that reductions in fitness in the wild environment are irrelevant.

Domestication as a source of new, complex and functional traits for the farm. The wild relatives of crops can be little more than genetic resources to be mined for novel alleles when only a few genes are likely to be successfully transferred to the domestic species by either introgression or transgenics. However, through domestication, entire biochemical pathways, plant organs or life history strategies are available.

Conceptually treating all domesticated grain species as a single ecological guild we can draw on the emerging field of landscape genetics (Manel *et al.* 2010) to suggest the value of dramatically increasing the genetic and phenotypic variation within grain crops by adding new species with new complex traits. Landscape variables play a vital role in conventional plant breeding as genotype x environment interactions determine the suitability of a particular crop or cultivar to a given region. While the aim of population genetics, a cornerstone of plant breeding, is the study of allele frequencies under various adaptive and neutral processes (Falconer and MacKay, 1996), landscape ecology aims to characterize the relationship between various environmental ecological processes and ecosystems (Ewers *et al.* 2013). The discipline of landscape genetics aims to examine how specific geographic and environmental factors shape population structure and can be useful in identifying traits of ecological significance. By extension ecological genetics is the specific study of these traits of ecological significance (Lesica and Allendorf, 1999) some of which are of interest to plant breeders.

Grain crop traits related to adaptation to novel habitats and abiotic stresses such as flooding and drought stress are increasingly sought after by plant breeders for the maintenance of yield in marginal or unpredictable environments. An ecological genetics approach may force breeders to look outside of conventional selection criteria because the domestication and later crop improvement of our current crops occurred under a particular set of "idealized" conditions; including growing regions with favourable rainfall or irrigation, fertile valley soils, monoculture cropping, and weed control. This process is inherently selective for genotypes—and species-- that do well under these conditions and not necessarily marginal landscapes. Many wild species on the other hand have undergone selection under extreme environmental conditions that do not normally present themselves in ideal agricultural systems and provide novel materials for crop development (Vear, 2010).

Domestication can now be accelerated. Advances in sequencing technology and statistical modelling are starting to eliminate barriers to the genetic dissection of complex quantitative and plastic ecological traits even in species which lack genomic resources or known pedigree (Sillanpää, 2011; Davey *et al.* 2011; Baxter *et al.* 2011; Narum *et al.* 2013). While important ecological traits such as nitrogen or water use efficiency may be the result of a combination of many genes each contributing small portions to the total variance in the trait throughout the genome, high molecular marker saturation is allowing for the development of whole genome breeding values and GS for complex traits using whole genome marker saturation. Whole genome GS using markers development techniques such as genotype-by-sequencing and RAD-seq have been effective in predicting selection candidates for complex traits such as grain yield in wheat (Poland *et al.* 2012) and are becoming more common in both plant breeding and ecological genomics (Narum *et al.* 2013).

II. Candidates for Domestication

Perennial sunflowers (*Helianthus* ssp., Asteraceae)

Annual sunflower (*Helianthus annuus* L.) is the only major grain crop domesticated in North America. The domestication of annual sunflower is believed to have been rapid as most of the traits which distinguish wild and domesticated plants such as achene and disc size are polymorphic or quantitative traits that show continuous distributions (Burke *et al.* 2002; Chapman *et al.* 2008; Blackman *et al.* 2011; Chapman and Burke, 2012).

Similar to annual sunflower (*Helianthus annuus* L.), the perennial North American species Maximilian sunflower (*Helianthus maximiliani* Schard.) and stiff sunflower (*Helianthus pauciflorus* Nutt.) have a range that encompasses most of North America, stretching from southern Texas to southern Manitoba. Both *H. maximiliani* and *H. pauciflorus* die to the ground each autumn surviving the winter through tuberous rhizomes. Similar to other wild sunflower species (including

wild *H. annuus*) they are self-incompatible outcrossers and the seed requires an extended (4-6 weeks) cool, moist stratification period for full germination.

H. maximiliani and wild *H. annuus* show clinal variation for a number of morphological and life history traits influenced by genetic drift and natural selection (Blackman, Michaels, and Rieseberg 2011; Kawakami *et al.* 2011). For example, Kawakami *et al.* (2011) found that Maximilian sunflower traits such as flowering time, disc size, plant height and growth rate exhibit a clinal distribution across a latitudinal gradient of North America. Discs ranged between 9.8-26.2 mm in populations collected from the southern range of the species in Texas to its Northern range in Manitoba.

The range of habitats in which these widely distributed perennial species persist leads us to predict that, like the wild annual sunflower, they harbour the genetic variation necessary for domestication. New mutations may not be required. Consistent with this prediction, we have found a great deal of variation for traits of interest within and between accessions collected from our locations in Kansas and Manitoba. Families collected across Manitoba grown under growth chamber conditions show considerable variation in plant height, days to flowering, disc size (3.8-18.3 mm), and branching architecture.

Helianthus maximiliani: Maximilian sunflower grows vigorously as a seedling, flowering in the first year. It has good seed fertility and well documented disease resistance including rust and *sclerotinia* (Rashid and Seiler, 2004; Muellenborn *et al.* 2011) and seed oil content equal or surpassing that of wild annual sunflower (Seiler and Brothers, 1999). The oil is rich in the essential fatty acid linolenic acid and higher in tocopherols (compounds with vitamin E activity) than any other wild Helianthus species evaluated (Velasco *et al.* 2004). As with many species of Helianthus interspecific gene transfer is possible though the viability and fertility of F1 interspecific hybrids is low (Atlagic *et al.* 1995). Chromosome doubling through colchicine has been proven to be effective in developing fertile *H. annuus* X *H. maximiliani* amphidiploids (Feng and Jan, 2008) and *H. maximiliani* has served as a source of novel restorer genes and male-sterile cytoplasms in *H. annuus* for hybrid breeding.

Maximilian sunflower is being domesticated simultaneously in Kansas and Manitoba. While genetic materials have been exchanged between these programs, the day length and climate differences make it probable that two distinct domesticated races are likely to emerge. In both locations the basic approach has been to collect seeds from a number of wild populations in the region of the breeding station, stratify seeds and transplant the seedlings to the field for evaluation. The program in Manitoba is still in the evaluation phase, although crosses have been made between wild Manitoba and improved Kansas individuals for the purpose of developing mapping populations. Initial results suggest that wild Manitoba populations are capable of persisting and will readily set seed in nutient deficient soils. Phophorus and nitrogen levels ranging between 2-16 ppm and 4-36 lbs/acre. respectively are capable of sustaining large stands

though the total number of seed bearing heads per plant are reduced under these conditions. Substantial variation in floral disk dimaeter, plant height and days to flowering have been observed between sub-populations in Manitoba suggesting that there is enough genetic variance to make genetic advancement for these traits within the population base.

In Kansas, selections from the initial evaluation nursery were intermated in 2003 to produce Cycle 1 plants. Selections from Cycles 1, 2 and 3 were intermated in 2006, 2009 and 20012 respectively. The general method has been to plant spaced-plant evaluation nurseries, select 20-50 individual plants using information about the performance of each plant's half siblings in the second year, dig the selected plants early in the third year and polycross them in a crossing nursery. For polycrossing, controlled pollinations were made using a bulk of pollen obtained by pooling pollen from the selected plants. Although many traits have been measured over the years, the selection indices were based primarily on seed per stalk, seed per head and average seed mass.

A side-by-side, multiyear comparison of the yield of selected Cycle 3 plants, random unselected Cycle 3 plants, and wild plants is currently in the field, so we cannot definitively estimate the gains made to date. However, heritabilities of the traits under selection (Table 1) and the data from each population (Figure 1), though grown in different years, suggest that yield has increased.

FIGURE 1. AVERAGE YIELD COMPONENTS OVER SEVERAL YEARS IN A *HELIANTHUS MAXIMILIANI* POPULATION UNDERGOING ARTIFICIAL SELECTION FOR INCREASED SEED YIELD

Each point on the graph shows traits from plants sampled in their second year (after transplanting). Experimental designs are summarized in Table 2. All experiments were established in Salina Kansas by transplanting clones or seedlings and were managed without fertilizer addition or irrigation beyond what was required to establish transplants. Weeds were controlled by cultivation except in the case of Experiment 2 (2005) where sunflowers were intercropped with alfalfa (*Medicago sativa*). The alfalfa and weeds were controlled by mowing between rows and within rows as necessary.
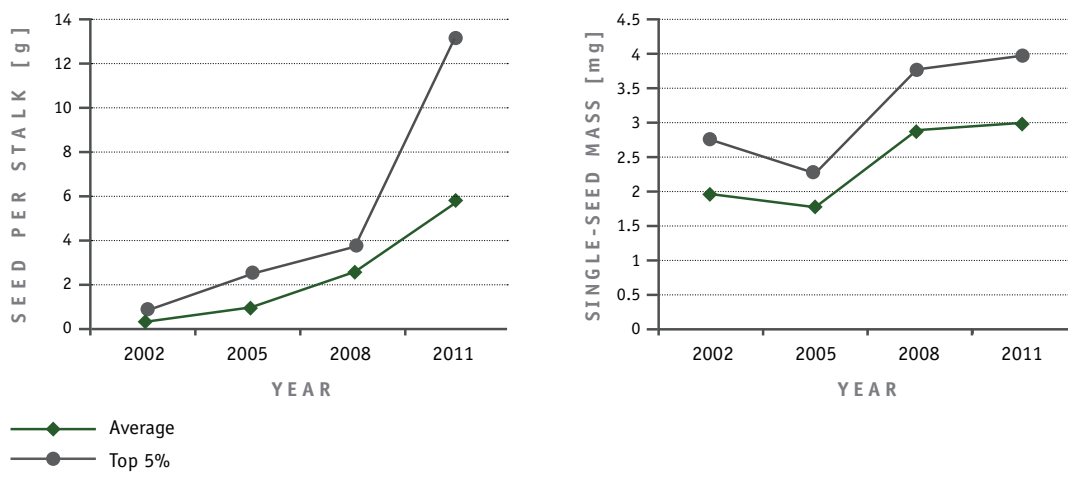
TABLE 1. MEANS, STANDARD DEVIATIONS AND RANGES, AND HERITABILITIES FOR SEVERAL TRAITS MEASURED IN SEVERAL DIFFERENT BREEDING POPULATIONS

Experiments were performed in different years and traits were not always measured the same ways, so only approximate comparisons are possible. Seedlings were germinated in controlled conditions and transplanted to the field in May. All experiments were performed in Salina, Kansas without fertilizer application or irrigation beyond the minimum required for transplant establishment. Only data from 2-year old plants is shown here unless otherwise indicated. Heritabilities were calculated from individual genotypes in known half-sib families using REML processes (Genstat, London) that included spatial components and blocking terms in the model only if they improved the model for a particular trait.

SPECIES, YEAR	NUM. PLANTS PHENOTYPED [NO. HALF-SIB FAMILIES]	SPACE BETWEEN ROWS (AND WITHIN ROWS) (m)	SINGLE-SEED WEIGHT (mg)			SEED YIELD PER STALK (g)		
			Her	Mean (stdev)	Range	Her	Mean (stdev)	Range
S. integrifolium 2008	2246 [224]	0.9 (0.9)	27	23.7(3.9)	12.0 - 42.5		10.9 (9.9)	0 - 83.1
S. integrifolium 2012	2720 [109]	0.9 (0.45)	61	15.6 (3.3)	5.6 - 29.5	27	3.46 (2.3)	0 - 19.9
H. pauciflorus 2012	2555 [100]	0.9 (0.45)	69	8.2 (1.7)	2.0 - 14.9			
H. maximiliani 2008	2240 [20]	0.9 (0.9)	27	2.86 (0.05)	0.42 - 7.68	14	1.33 (1.07)	0.01 - 12.9
H. maximiliani 2011	710 [50]	1.8 (1.8)	34	2.98 (0.80)	0.407 - 15.7	42	5.74 (3.72)	0.01 - 36.9
H. maximiliani 2012*	1925 [113]	0.9 (0.9)	49	4.88 (0.75)	3.33 - 7.65			

* Data taken in first season on a subset of plants, selected visually for reduced branching

	SEED YIELD PER HEAD (mg)			SEED YIELD PER HEAD AFTER SHATTERING (mg)			PERCENT NOT SHATTERED			OTHER TRAIT OR NOTES			
	Her	Mean (stdev)	Range	Her	Mean (stdev)	Range	Her	Mean (stdev)	Range	Trait	Her	Mean (stdev)	Range
	13	423 (142)	0 - 992							Stalk diameter		9.78 (3.6)	2 - 20
										Num ray florets per head	78	51.8 (13.6)	8 - 165
	71	291 (197)	0 - 135	67	107 (82.2)	0 - 641	42	43.3 (22.0)	0 - 100	Seed not shattered (%)	42	43.3 (22.0)	0 - 100
	42	415 (122)	14 - 844		35.0 (27.1)	0 - 824	16	10.74 (7.46)	0 - 100	Number of stalks per plant	16	19.4 (8.97)	2 - 80
		206 (112)	3.17 - 554		12.2 (13.1)	0 - 153.5	51	9.56 (12.9)	0 - 96.8	Number of stalks per plant	48	24.3(11.0)	3 - 75
	31	640 (362)	0 - 2240	33	296 (199)	0 - 1470		48.9 (20.3	0 - 100				

In 2007, a single unusual individual was observed in the Cycle 2 evaluation nursery. This plant had strongly reduced branching and a single, larger than average head on each main branch. Open pollinated progeny from this plant were intermated in 2008 to produce a segregating population in 2009. Plants with strongly reduced branching in their first season were intermated that same year and this process was repeated in 2010 and 2011. Data from the initial reduced branching segregants (2009) show reduced seed mass per head thereby confirming our observation of malformed heads and florets (Figure 2). By 2011 the average yield per head was approximately equal to that of the population from which the first reduced branching plant was taken in 2007, and some individuals had yields per head exceeding anything in the normally branching population, again, confirming our observation that reduced branching is associated with a wider range of disk diameters. We attribute the restored seed fertility to natural selection in which more fertile individuals contributed more pollen and more seeds to the subsequent generation than less fertile individuals. Reduced branching materials are currently being crossed with wild-type materials from Manitoba to develop mapping populations and to introduce this trait into a northern adapted genetic background to expand the diversity of the uniheaded lines and develop divergent breeding pools.

FIGURE 2. QUANTITY OF SEED PER HEAD HISTOGRAMS SHOW CHANGES IN A *HELIANTHUS MAXIMILIANI* POPULATION UNDERGOING ARTIFICIAL SELECTION FOR INCREASED APICAL DOMINANCE

Heads were sampled in the same year as seedling establishment. The 2007 sub population is the same as the population shown in Figure 1. Open pollinated progeny of a single unusually large-headed, nearly unbranched plant in that experiment were intermated to produce the sub-population evaluated in 2009.
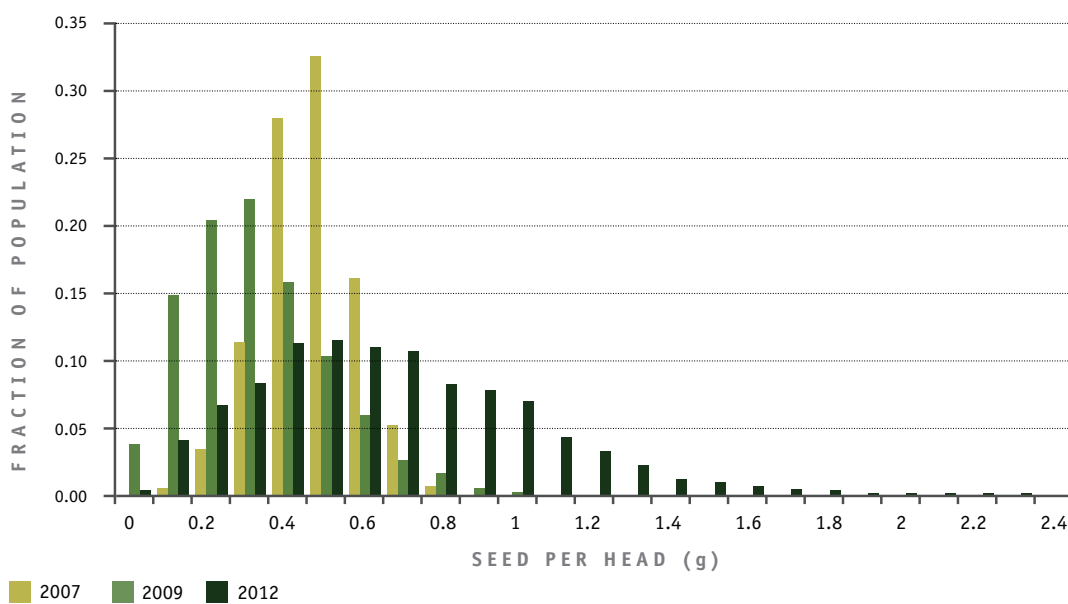
TABLE 2. EXPERIMENTAL DESIGNS

EXPERIMENT	YEAR PLANTED	YEAR DATA TAKEN	EXPERIMENTAL FACTORS			SELECTION		NOTES
			Plots	Number of families	Number of genotypes	Num. of genotypes selected to advance	Percent of genotypes selected to advance	
1	2001	2002	960	96	960	150	15.6	Families are batches of seeds collected from different wild populations
2	2004	2005	900	15	150	20	13.3	Genotypes in this experiment are a subset of the genotypes in the first experiment. Selected individuals were dug and their rhizomes divided and transplanted to the new nursery with clonal replication
3	2007	2008	2240	20	2240	50	2.2	Selected plants from Experiment 2 were intermated to produce seed for Experiment 3
4	2010	2011	710	50	710	50	7.0	Selected plants from Experiment 3 were intermated to produce seed for Experiment 4

Helianthus pauciflorus (= H. rigidus)* and *H. tuberosus: these are tuberous, hexaploid perennial sunflower species (n = 51). We consider them together here because they are known to hybridize naturally forming fertile populations (Brooks and Schofield, 1986) that have previously been given species status and are now considered subspecies or hybrids. Clevenger and Heiser (1963) commented that "In their extreme forms, the [hexaploid *Helianthus*] taxa considered here are quite distinct, but intergradations are frequent in nature." In Kansas, *H. pauciflorus* and *H. tuberosus* are readily distinguished when collecting seeds or plants from wild populations. However, open-pollinated progeny of wild-collected individuals grown in a common garden are much more difficult to categorize than their parents, presumably due to interpollination.

Both tuberous species were used as parents in interspecific crosses with *H. annuus,* mainly between 2004 and 2009. *H. pauciflorus* gained attention as a candidate for direct domestication because some individuals had larger seeds than *H. maximiliani* and large heads bourn on long peduncles. The branching pattern and peduncle length place the infructescences largely above the leafy portion of the plant, making mechanical harvesting easier. Although The Land Institue

population under consideration for perennial oilseed development is referred to as *H. pauciflorus* it could more accurately be considered a hybrid swarm with individuals positioned all along the intergradation between the two wild hexaploid species.

Both tuberous species are sources of resistance to rust (*Puccinia helianthi*) (Zimmer and Rehder 1976), and both are crossable with *H. annuus*, though, as described above, the hybrids are only weakly fertile (Georgieva-Todorova, 1964; Jan, 1997). Despite the barriers to gene flow, *H. tuberosus* has been the source of important disease resistance genes in *H. annuus* (Rogers *et al.* 1982), and *H. pauciflorus* has provided cytoplasmic male sterility genes (Jan *et al.* 2002). The cytoplasm of many commercial sunflower cultivars may actually be from *H. tuberosus* (Jan and Ruso, 2000). *H. tuberosus* was domesticated as a tuber crop and both tubers and vegetation could be used for bioethanol production or livestock fodder (Kays and Nottingham, 2007). *H. pauciflorus*, though never domesticated, is also strongly tuberous.

The yield potential of this species remains unknown, though initial field trials were established at the Land Institute in 2011, the University of Manitoba in 2012, and Organic Valley Co-op (Cashton, Wisconsin) in 2013 to evaluate this species. Perhaps because of the large phenotypic variation resulting from the intergradation between the two species and their respective hexaploid genomes, the heritabilities for traits of interest measured in a large Land Institute evaluation nursery in 2012 were high (Table 1), suggesting that a breeding program could be successful. However, the extreme sensitivity of sexual reproduction in this population to drought led the Land Institute to phase out its evaluation of this species in Kansas, focusing efforts on the collaborative breeding effort in Wisconsin where this species may be better adapted to the shorter growing season and lower potential evapotranspiration.

Helianthus cusickii: Cusick's sunflower is a diploid perennial (2*n*=34) adapted to dry, rocky soils of high mountain desert, surviving with annual precipitation of only 25-60 cm (Seiler, 1992) by means of a robust taproot. It flowers earlier in the year than the other perennial sunflowers discussed here and is noted for morphologically being the most extreme species in the genus (Heiser, 1965). Counterintuitively, it is more closely related to *H. annuus* than the other perennial species that have been used as sources of genes for the improvement or perennialization of *H. annuus* (Timme *et al. 2007)*. However, both pre and post zygotic barriers to gene flow are expected (Sideli *et al.* 2013). We have made preliminary attempts to produce interspecific hybrids between *H. cusickii* and *H. annuus*. Putative hybrids are being observed and additional attempts at hybridization will be made and it remains to be seen if genes for perenniality, fleshy taproots, or other drought tolerance traits can be introgressed to the annual crop sunflower.

Its early flowering, small pubescent leaves, modest height and deep roots make this species a possible candidate for domestication as a drought tolerant oilseed, especially if domestication related genes could be introgressed from *H. annuus*. However, after planting out hundreds of individuals representing a large portion of the species' native range (seeds collected in California,

Nevada, Washington and Oregon) in typical agricultural soils in Kansas, we found poor survival and low vigor compared to several other annual and perennial *Helianthus* species. Possibly this species has not been exposed to soil pathogens typical to agricultural soils but uncommon in desert soils. However, a few individuals did survive into a second year and, if intermated, could be used to develop a population with greater adaptation to agricultural conditions.

CANDIDATES FROM OTHER GENERA

While the genus Helianthus is an obvious source of perennials that could be domesticated as oilseeds because it includes numerous perennial species as well as two domesticated species (*Helianthus annuus* and *Helianthus tuberosus*), the North American grasslands contain many other species with oil rich seeds. Some of these are likely to have chemical or anatomical adaptations not available in *Helianthus*.

Silphium **(Asteraceae):** The species of the genus *Silphium* have much larger seeds (e.g. 21 mg reported by Kowalski and Wiercinski, 2004) than most other native North American forbs and grasses. The flavor and composition of major nutrients is similar to that of the domesticated sunflower (Kowalski and Wiercinski, 2004). The heads resemble that of a sunflower, but whereas the disk florets of *Helianthus* are perfect and the ray florets are sterile, in *Silphium* the ray florets are pistilate and the disk florets are staminate (Brooks and Schofield, 1986). Several observations of wild stands of *Silphium integrifolium*, a fairly common prairie species in Kansas, led us to consider this species for domestication. In addition to the size of its seeds, we observed variable seed shattering. Mature seeds remain on some heads well into the autumn or even the winter. This is easy to see because the large seeds remain attached to the heads after other floral structures and sterile florets have fallen off. The other key observation was the performance of this species during dry intervals or even serious droughts. In 2012, the drought in Kansas was severe enough that test plots of commercial hybrid sunflowers administered by Kansas State University in nearby Hutchinson, Kansas, were terminated due to crop failure (Stewart Duncan, personal communication). The average seed yield of S. integrifiolium in The Land Institute breeding nursery, where plants had been transplanted the year before in 0.9 m rows, 0.45 m within rows, with no fertilization, was 312 kg/ha and some exceptional plant were estimated to have yielded up to 1182 kg/ha.

In 2001-2002 we evaluated both *Silphium integrifolium* and *S. laciniatum*, another drought tolerant, perennial species, for their adaptation to agricultural conditions. We found that neither species flowers in the first year. They produce large basal leaves and no stems. In the second year *S. integrifolium* reliably produces stout stalks and flowers. *S. laciniatum* is much slower to develop and does not always flower in the second year. We judged its establishment phase to be too slow for agriculture and focused on *S. integrifolium*.

The most obvious barrier to high seed yield in all *Silphium* species is the production of seeds by only the ray florets which number from 15-32 per head in *Silphium integrifolium*. To test whether genetic variation existed for this trait, and whether the number of fertile florets could be increased to at least 100 per head, we practiced recurrent selection for that trait alone. Seed from 21 Kansas populations were allowed to interpollinate in 2004. Approximately 100 000 progeny were planted in 2005 and screened in 2006. Because scoring the number of ray florets is very time consuming, we never measured the population average thoroughly, but estimated it to be about 28-30. The ray florets on plants visually identified as having heads with high numbers of ligules (the long, strap-like corollas of ray florets) were carefully counted. Eighty three plants with 38 or more ligules were found and intermated that year. The progeny of this polycross were planted in 2007 and similar recurrent selection and intermating was done in 2008 and the process repeated in 2009-2010. In 2012, finding that the average number of ray florets in a population of 2 720 was 52, that several plants had over 100 ligules and that one had over 150, instead of simply intermating the plants with the most ray florets, we decided to make selections using an index based on seed yield per stalk and seed size (mass). We reasoned that we had good evidence that the population contained adequate genetic variation for ray floret number and that pushing the population mean higher prematurely might result in loss of variation for seed size or other traits of interest. Furthermore, some of the plants with very high ray floret numbers are now close to being male sterile.

Linum **(Linaceae):** Lewis flax (*Linum lewisii* Pursh.) and stiffstem flax (*Linum rigidum* Pursh.) are both perennial North American species in the same genus as domesticated annual flax (*Linum usitatissimum*). Antinutritional saponins are reported in *L. rigidum*, limiting its usefulness to agriculture. Cultivars of *L. lewisii* with improved longevity, drought resistance and disease resistance have been developed as ornamentals and for use in landscape reclamation. This species also has good forage value.

L. lewisii has fewer chromosomes (n=9) than annual domesticated flax *Linum usitatissimum* L. (n=15) which could pose a barrier to interspecific hybridization. Fortunately unlike the large complex genome of polyploid candidate perennial grain species (perennial wheat ~17 Gb) or those with highly repetitive genomes (*Helianthus* spp. ~3.5 Gb) the flax genome is small (~370 Mb) and whole genome resequencing is emerging as a technique for improving domesticated flax (Fu, 2012). The small genome size in the genus *Linum* in theory should facilitate whole genome association studies into the genetics of domestication and for screening germplasm for favorable traits.

III. Discussion

Assuming that we have successfully argued the case for domesticating perennial oilseeds, we (authors, scientific community and society) are still faced with difficult decisions about which candidate species are most promising.

The candidates from the Asteraceae, described above, provide an excellent study in contrasts between "perennials." Whenever we have done the experiment, we have found that the traits of interest are heritable. It is difficult—maybe impossible—to judge which species has the highest yield potential, given unlimited cycles of selection for yield. We argued above that it might be simpler to domesticate species by directional selection on quantitative traits like yield and (in sunflowers) shattering than to attempt to introgress complex traits such as lifespan. The same logic may apply within the subset of perennial species. Root structure or vegetative spread by tubers may involve complex developmental pathways. Other traits may simply be very expensive to measure due to the instrumentation required or need for controlled/multiple environments. It would be well to consider the following traits carefully when selecting a perennial species for further investment.

ABOVE-GROUND PLASTICITY

To our surprise, we discovered that *H. pauciflorus* and *H. maximiliani* plants when dug from a mature prairie and transplanted to an experimental plot were likely to triple or quadruple in height. Leaf size, stem diameter and number of inflorescences are also altered in this environment. In short, the plants look like a different species. One implication of this phenomenon is that it is impossible to judge the agricultural phenotype of a wild species without growing it in rows or as spaced plants in an experimental plot where they have a different nutrient status and are released from competition with other plants. A more subtle implication is that we have found that plants with this level of plasticity are highly sensitive to both drought and self-crowding and may require nutrient limitations to modulate overabundance of growth. A positive implication may be that these species are capable of producing seed under resource poor conditions.

Domesticated sunflower has a high rate of evapotranspiration, a trait often exploited as an ecosystem service to lower the water table in areas dealing with salinity. Under drier conditions this can result in drought stress later in the growing season greatly impacting yield potential because the reproductive growth stages of sunflower are more sensitive to moisture stress than the vegetative stages (Göksoy *et al.* 2004). In the southern part of its range (e.g. Kansas), Maximilian and stiff sunflower undergo an extended vegetative period beginning with emergence in May, expend soil moisture and accumulate biomass throughout the season. If moisture or fertility is available early in the season, plants can grow quite tall and branch freely. Frequently as temperatures rise throughout the season these species often endure mid-season drought resulting in a loss of accumulated biomass and potential volatization of nutrients prior to reaching its necessary short day photoperiod requirement for flowering in September.

Thus the plasticity of *H. maximiliani* and *H. pauciflorus* cause—from the perspective of maximizing seed production per unit area—excessive vegetative growth early in the season

and excessive vegetative growth in high rainfall years. Both problems result in reduced harvest index (if abscised leaves are included in the calculation) and premature depletion of soil moisture and potential for nutrient volatization. A second issue is that many of the phenotypes of interest to breeders are likely to respond to the environment in ways that are difficult to quantify, impeding breeding progress. For example, small scale patchiness in soil texture or drainage within a breeding nursery can be difficult to model using conventional blocking or even spatial statistics. Neighboring plants also modify each other's environments in ways that are difficult to control or model especially early in the domestication process when the phenotypic variance of the population is great, when gaps are produced irregularly in the field due to plant mortality, or when transplanting or cultivating errors create slight differences in the spacing between or within rows.

In contrast, *H. cusickii* and *S. laciniatum* showed only modest, if any, increase in growth or stature when grown with reduced competition. Unfortunately, this makes them very uncompetitive with weeds, at least in their first few years, and therefore difficult to work with. *S. integrifolium* has an intermediate response growing about twice as tall in cultivation than in the wild. This species also appears to have much greater seed yield and height stability from year to year. One reason for its reduced plasticity is that like the other species it ceases to grow in height at the time of flowering but unlike the Helianthus species, in Kansas it flowers beginning in early July whereas *Helianthus pauciflorus* begins flowering in late August and *H. maximiliani* in early September.

This gives us a clue about a strategy for reducing the plasticity of the *Helianthus* species in the southern part of its range: introgress flowering time genes from points further north. Manitoba *H. maximiliani* populations flower in July.

RHIZOMATOUS SPREADING

S. integrifolium is less plastic than *H. maximiliani* and *H. pauciflorus* in another way: it forms a crown that does not increase in diameter much over time. This means that the number of stems per unit area increases very slowly, if at all (after the establishment year). At the other extreme, *H. pauciflorus* produces rhizomes that radiate 30-50 cm from each stem in all directions. The number of stems per unit area increases geometrically from year to year, leading to severe crowding and stunting of the stems. Without thinning, by the third season, the plants are as short and produce as few heads as when this species is found growing in a mature prairie.

Aggressive asexual spreading impedes plant breeding progress because adjacent genotypes in a nursery are usually impossible to separate in the second season when they are regrowing from large overlapping rings of rhizomes/tubers. This makes it difficult and risky to make selections of individual plants based on their reproductive traits from the year before. Instead, a subset of individuals in a segregating population would be visually selected and their tubers

dug before the rhizomes connecting them to the original seed-grown plant rot. Tubers would be planted in small plots and genotypes selected for intermating on the basis of plot yield. Although this digging of tubers and establishment of plots of clones would be labourious and eliminate the possibility of making selections the first year after intermating, some traits could be measured this way that cannot be measured on individual spaced plants (see the Tubers section below).

A more serious problem is the phenomenon of stand decline. *H. pauciflorus* stands decline in seed yield rapidly after the first year. As described above, as stalk density becomes greater, the stalks become shorter and more slender. Many completely fail to flower and those that flower produce much smaller heads. We have not studied the effect of ramet density on root traits, but we hypothesize that roots also become shorter and more slender. The "luxury uptake" of water by such dense stands early in the season may be another factor in stand decline. A second-year population in Kansas, 2012—a dry year, declined so strongly that by midsummer most stalks were extremely stunted and had only brown leaves. In contrast, *S. integrifolium*, planted in the same field at the same time grew and flowered normally.

TUBERS

The tubers of *H. pauciflorus* and *H. tuberosus* are a curse when trying to achieve and maintain an optimal plant density, as described above, but also a potential blessing. Although asexual reproduction of this kind would require farmers to diligently cultivate between and possibly within rows to maintain stalk density within target range, it would also provide an easy method for propagating genetically uniform cultivars, bypassing the need for years of inbred line or synthetic cultivar development. The tubers are easy to dig, store and transport, fairly uniform in size, and could be planted with standard potato planting machinery. Possible applications of tuber propagation:

1. Fixed specific combining ability: Fortuitous crosses creating even a single outstanding F1 individual could be all that is required to instantly create a superior genetically fixed cultivar despite chromosome compliment. Emasculations and careful pollination would not be required to generate propagules each year. And unlike potato, where micro-tubers are sometimes produced in vitro to reduce the costs of shipping whole field-grown tubers or cutting them into pieces, the tuberus rhizomes of *H. pauciflorus* and *H. maximiliani* are already small.

2. Locally adapted cultivars: Ecovars [TM]—improved ecotypes—have been proposed as a way of exploiting the adaptations that have evolved in local populations of a species. Attempting to produce numerous locally adapted cultivars of an outcrossing species, while retaining hard-won domestication traits and population uniformity in phenology and plant height, is a daunting prospect. Paradoxically, the possibility of eliminating genetic variation at the field

scale through asexual propagation may make it more feasible to release numerous genetically distinct locally adapted cultivars.

3. Ecological genomics research: Ecological genomics aims to 'find the genes that matter', that is those who influence adaptive variation in the context of their natural environments (Edwards 2013). Being able to propagate individual genotypes asexually (and inexpensively) would allow new phenotypes to be mapped in segregating populations or diverse association mapping panels. The phenotypes of most interest to agronomists, yield, water use, nutrient uptake, stalk density over time, are so easily confounded by inter-genotype competition and plant spacing, that they can not be meaningfully measured on individual seedling-grown plants. These traits can be measured using inbred families derived from segregating populations, but this is much more difficult to accomplish with outcrossing species and likely to be confounded by inbreeding depression therefore association as opposed to pedigree based approaches may be better suited for these species.

ROOTS

Belowground traits are difficult to observe and, therefore, difficult and expensive to change directly using conventional phenotypic selection. The range of root traits among seed-producing perennials is very great. In selecting between candidates for domestication as new crops, it would be wise to carefully consider which kind of root system is needed to provide the desired ecosystem and economic services in the target environment. We have speculated that with a few exceptions (wheat, sorghum) it may often prove to be easier to domesticate species in order to capture their life history and cold tolerance traits (perenniation) than to try to breed or engineer such complex traits in an annual crop using de novo variation or variation brought in from wild relatives. If this logic applies to the easily scored trait of winter survival, it is applies more strongly for the possibly equally complex rooting traits which are far more expensive to "phenotype" under realistic field conditions.

In contrasting the response of experimental populations of *Silphium integrifolium*, *Helianthus maximiliani* and *H. pauciflorus* to drought it is impossible to separate the effects of root depth and increasing stem density. Adjacent, same-age plots of *S. integrifolium* appeared unwilted throughout the 2012 growing season while in midsummer, *H. pauciflorus* appeared scorched and green material was only seen after autumn rain and lower temperatures. The response of *H. maximiliani* was intermediate. The plants were shorter than normal, many leaves dropped, and the remaining leaves were permanently wilted during July and August. A counterintuitive aspect of these observations is that *H. pauciflorus* is often found in dry, rocky, shallow-soil locations, while *S. integrifolium* is often seen at lower landscape positions where soil and moisture are more abundant.

Weaver and Stoddart (1935) noted similar contrasts between *H. pauciflorus* and *S. integrifolium* during the drought of 1934 (the "dust bowl" years), writing, "A few species such as ... *Silphium integrifolium*, grew normally and stood unwilted above the dried grasses." They attributed the drought avoidance of these forbs to their deep roots: "Among the forbs, resistance to drought was closely correlated with root extent. Species with root systems penetrating 8 to 20 feet into the moist subsoil were little affected."

These authors also noted that, "*Helianthus rigidus* [= *H. pauciflorus*] was often completely wilted and the leaves dried [in the prairie] while a foot or two distant in cultivated land the same species was 3 feet or more tall, abundantly supplied with flower heads and completely turgid." This statement is consistent with our observations of great differences in height, number of heads and drought stress between seedlings ("cultivated land") and second or third year stands in which the plant density approaches that of the prairie and presumably results in soil water depletion. The authors clearly characterize this species as drought tolerant, stating that "the tenacity with which [*Helianthus rigidus*] persisted after weeks of semi-wilting was impressive."

In climates experiencing variable rainfall within and between growing seasons, the advantages of drought avoidance through persistent deep root systems over mere drought survival through osmotic adjustment, wilting, and stunting seem obvious. For subsistence agriculture, yield stability is more important than yield potential, and crops that yield well during dry years would increase food security.

Increasing the rooting depth of crops has been proposed as a method for sequestering meaningful amounts of carbon (Kell, 2011). If deep roots persist from one year to the next (low turnover) they may not require much more photosynthate for maintenance than shorter root systems. However, in the first few weeks or months of life, the construction of this root system will almost certainly require greater allocation of photosynthate belowground than for other types of root systems. The cost, then, is likely to be reduced aboveground growth in the first year. This translates to poor weed competition (*Silphium* spp. and *H. cusickii*) and/or no flowering in in the first year (*Silphium* spp.). A less obvious side effect of deep persistent roots is reduced seedling nutrient and water acquisition. The surface area of a few large-diameter seedling roots heading almost vertically downwards must be lower than a finer more branching seedling root system. We have seen that following transplanting *S. integrifolium* seedlings are more vulnerable to water stress, experiencing higher mortality in unirrigated plots, than perennial *Helianthus* seedlings. Thus long-term drought avoidance comes at the cost of poor drought tolerance, especially in the first few weeks.

We do not know the costs or benefits of the various rooting strategies in other circumstances. In locations with poor drainage or frequent floods, are fast-growing "disposable" root systems a better investment than costly, deep, permanent ones? Which root systems tolerate root predation better? Do root systems with large root systems and low root turnover tend to immobilize soil nitrogen leading to nitrogen deficiencies?

APICAL DOMINANCE

Dramatic changes in aboveground branching have happened during the domestication of many crops including annual maize and sunflower. So on the one hand, the branching pattern of a wild plant need not eliminate it from consideration for domestication. On the other hand, branching in perennials is much more complex than in annuals. Herbaceous perennials regulate their architecture by controlling branching and internode length at several points including the below-ground branches (rhizomes, tillers), main lateral branches on stalks, and short flowering branches.

As described above, *Helianthus maximiliani* families with a single head per stalk are now available, as are multiheaded types and intermediate lines that produce only a few short flowering branches just below the terminal inflorescence. Increased apical dominance resulting in fewer, larger infructescences and resulted in the characteristic large head of domesticated sunflower. Depending on the context of the habitat in question apical dominance is observed to both positively and negatively impact relative fitness (in the form of intact harvestable seed) in annual sunflower (Reagon and Snow, 2005). The development of a single determinant head increases seed size but can leave the plant vulnerable to seed predation or head loss by the native head clipping weevil. Reduced branching and number of sub-terminal inflorescences on the main stem increases both the synchrony of head maturation and the uniformity of head position on the plant. Both facilitate harvest and reduction in losses to seed predators and shattering. Synchrony of anthesis and maturation means that mature heads do not need to be left in the field—where they are exposed to shattering and bird predation--while immature heads continue to develop. Positioning heads near the top of the plant means that less leafy material must be processed by mechanical harvesters. Excessive foliage near the heads can slow the harvesting process, require suboptimal winnowing settings, or result in contamination of the harvested seed by wet material.

In general, apical dominance combined with early flowering may present an opportunity for increasing the harvest index of perennial oilseeds while reducing the phenotypic plasticity. The longer growing season of perennials compared with annuals allows them to produce more biomass, but in opportunistic, highly indeterminate species, even if stems were harvested as a co-product with the seeds, much of this productivity may be unharvestable (dropped leaves, withered, headless stems, aborted heads) in a dry year or present harvesting difficulties (thick woody stalks, excessive wet, green material) in a good year. Where oil production is the main goal it is clear that either the plants must stop growing vegetatively and enter the sexual phase early in the growing season or that the plants must be low-growing early in the season, accumulating carbohydrate reserves belowground and then bolt late in the season, producing heads on largely leafless stems.

While in theory breeding *H. maximiliani* to flower earlier in the season in the southern part of its range could accomplish these goals, it remains to be seen whether this will succeed. One possibility is that after flowering, apical dominance will be released and new primary stalks may emerge from the rhizomes or the existing stalks may continue to branch and produce leaves without a means of accumulating reserves for the following year. Another possibility is that the plant may simply die back. This would accomplish the goal of conserving soil moisture for the next season, but would reduce the productivity of the cropping system in good and average years. Both of these phenomenons have been observed under growth chamber conditions suggesting possible genetic variance for the regulation of rhizome dormancy following flowering and could potentially be manipulated as well. In the case of tuberous perennials, early flowering may help reduce the harvest index, but the opportunity for storing carbohydrates after seed filling is limited. This is because the main sink at this point is the new tubers, but only a single replacement tuber is required to maintain the stand density and excess tubers may need to be removed to prevent overcrowding. For regions with short growing seasons, these points may be irrelevant. Water stress may be a rare problem and winter imposes a limit to excessive vegetative growth. This may be the only suitable environment for tuberous perennials.

SENESCENCE

One of the most useful features of the annual habit is the programmed death of the stems and leaves bearing the heads to be harvested. Senescence allows the grain and the stem to dry prior to harvest. While the individual heads of the native oilseeds surveyed here senesce when the seeds are mature, the stalks and leaves generally do not until killed by frost. At that point, the weather is cool and the plant material dries slowly. Opportunities for remobilizing biochemical contained in the leaves and stems are also missed. Conversely under growth chamber conditions following flowering senescence has been observed in plants despite optimal temperature, moisture and nutrient conditions suggesting a killing frost may not be necessary to trigger senescence.

If candidates with autumn foliar senescence are not found, chemical defoliants--already used to facilitate dry down and harvest of some annual grain and perennial hay crops-- could be used with perennial oil seeds. An alternative would be to look for species that produce most of their leaves close to the ground and send up a specialized stem with fewer or smaller leaves to display the inflorescences above the canopy. Even if this stem did not senesce after seed maturation, it might be less succulent and leafy than the stems of other species and thus present fewer problems for harvest. Some prairie plants produce such structures, including *Tripsacum dactyloides*, *Silphium laciniatum* and *S. terebinthaceum*. Perhaps these *Silphium* species should be re-evaluated as potential oilseeds. It would be necessary to demonstrate that such a species

also had genetic variation for increasing the diameter and rigidity of such a stem in order to bear the weight of larger, heavier seed heads.

Plant senescence in annual crops also gives the farmer a high level of control over the plant density each year. We alluded above to the potential problem of ramet proliferation through rhizomatous and tuberous asexual propagation. Even when the fine roots or leaves of perennials senesce, shoot and root apical meristems may not. With woody crops, pruning to control the number of aboveground meristems (buds) is crucial for maintaining fruit size and plant productivity from year to year (Marcel-les-Valence, 2006). In the absence of programmed meristem population control, agronomists may need to develop methods to "prune" back the above and below-ground meristems of perennial herbs.

The predictable senescence of all the plants in a field of an annual crop provides an opportunity for tillage or pesticide application to kill any invading vegetation and to disrupt the lifecycles and habitats of many kinds of agricultural pests. Of course, the downside of this is that the soil is exposed to erosion and the habitat of beneficial or threatened animal species is compromised. How can perennial grain farmers achieve the necessary function resetting secondary ecological succession annually without the options available to annual grain farmers? Controlled burning and grazing have been used for centuries to arrest succession at a point that provides abundant palatable herbaceous species for grazing livestock. Both of these interventions can prevent the invasion of woody plants that lose their competitive advantage when their aboveground meristems are killed each year. However, many kinds of perennial or biennial weeds are herbs and tolerate these stresses necessitating a diversified integrated approach. We have found that improved populations of perennial oilseed candidates, once established are quite competitive with other herbs and prevent their invasion. Breeding to increase determinacy, advance flowering in the season, reduce asexual reproduction, and increase harvest index is likely to make these species less competitive with other herbs. Perhaps this is an ecological tradeoff. An agroecosystem in an un-invadable state is also in a state in which asexual structures and processes dominate. The *H. pauciflorus* population at The Land Institute in 2013 illustrates this. The density of ramets has increased each year since seedlings were transplanted in 2011. The rows have completely disappeared but no cultivation has been practiced for two years and yet it is a single species monoculture. On the other hand, the plants are nitrogen and moisture stressed and very few, tiny heads were produced.

Maintaining perennial forbs in rows and lightly cultivating between the rows early in the growing season may be one way that farmers can prevent overcrowding yet control invading perennials. Alternatively integrating animals which exhibit root scavenging behavior such as pigs in between rows may be one way of reducing unwanted spread while transporting nutrients across the landscape. If it is necessary to thin (and then weed) the stand within the row also, specialized cultivators or a companion cover crops may need to be developed.

CONCLUSION

Before investing heavily in new perennial crop domestication for a particular commodity and target environment, it would be worth thinking very strategically about what traits other than multi-year survival need to be captured. The breeders should begin discussions with agricultural engineers and agronomists to develop new kinds of equipment and procedures to enable farmers to fulfill the functions that the candidate species cannot do itself. The integration of companion species, equipment capable of root/rhizome "pruning", strip tillage and possibly mowing or cultivation within rows may prove to be necessary in many cases. At a more philosophical level, we wonder if it is possible to predict *a priori* what histories of natural selection are likely to have produced species with complex traits compatible with perennial agriculture.

Hypothesis 1: The life forms that dominate the native ecosystem in the regions and landscape positions targeted for grain production serve as useful models but may themselves not be useful for domestication as grains. Once a particular kind of life form (e.g. warm-season, sod-forming grasses in Kansas) outcompetes other life forms the major source of competition is intraspecific or at least intra-guild. This kind of competition leads to "arms races" which lead to the evolution of highly competitive morphologies such as trees with long trunks and prairie grasses with massive root systems (Craine, 2006). Undoing the effects of these arms races to produce a more efficient canopy or root community could be very difficult.

Non-dominant species have had to evolve strategies for escaping direct competition with the dominant life forms. Thus, in Kansas prairies, deep rooted forbs in the absence of disturbance may never be able to outcompete sod-forming grasses and become dominant, but they can survive as dormant tubers or seeds until a serious drought kills some grasses opening up niche spaces for new growth. The deep-root lifeform may be more useful in the context of agriculture in Kansas than the warm-season sod forming life form. On some landscape positions in this biome, *H. pauciflorus* and *H. maximiliani* can form large stands that are co-dominant with some grasses. *H. maximiliani* and *H. tuberosus* have been described as having allelopathic properties, this coupled with abundant adventitious growth in the presence of available resources tells us these species are highly competitive through a series of diverse mechanisms. However, in different environmental contexts, these species may never be able to form dense stands and thus may not be as competitive with other species...or itself.

Hypothesis 2: Climates or landscape positions where neither perennial nor annual agriculture is possible might seem to be sources of species well adapted to the kinds of stresses perennial crops in moderate-to-low input systems are likely to face. However, we suggest that species found mainly in these extreme locations may have experienced selection for stress tolerance rather than stress avoidance. Reduced growth rate is one way of surviving in a stressful environment, but this strategy is likely to be inimical to any form of agriculture. Evidence for this comes

from switchgrass (*Panicum virgatum*) where cultivars derived from the wild upland ecotype showed less physiological stress under water and nitrogen limitations than lowland cultivars, but nevertheless were out-yielded by the lowland types in both stressed and unstressed treatments (Stroup *et al.* 2003).

Another strategy that approaches stress escape (fully realized in the annual habit) is highly plastic development. Such plants may be diminutive and asexual most years but capable of opportunistic growth and profuse flowering following disturbance or in favorable seasons when limitations have been temporarily relaxed (c.f. description above of *H. pauciflorus* in sod versus tilled ground). Stress tolerance/escape adaptations enable a species to survive but may not be useful in an artificial ecosystem designed to both take full advantage of available resources and to minimize seed yield instability from year to year and over the course of centuries.

The ideal perennial grain has adaptations for avoiding water and nutrient limitations, for example by exploiting deep soil moisture unavailable to other species, or by interacting with mutualists to obtain nitrogen or phosphorus in forms usable by plants. We suggest, therefore, that the best candidates for a region may be those capable of persisting and fruiting annually (though perhaps not as the dominant life form, according to Hypothesis 1) in the absence of agricultural inputs or nutrient-releasing disturbance but with the benefit of the soils and landscape positions where perennial agriculture is possible to be practiced.

These hypotheses are suggested as criteria for quickly identifying initial candidates and their adaptations to their environmental constraints; there is still no substitute for rigorous evaluation of all candidates for adaptation to the target range of stresses and input levels.

REFERENCES

Agarwal, A.K. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in energy and combustion science*. 33(3): 233-271.

Atlagić, J., Dozet, B. & Škoric, D. 1995. Meiosis and pollen grain viability in *Helianthus mollis, Helianthus salicifolius, Helianthus maximiliani* and their F1 hybrids with cultivated sunflower. *Euphytica*. 81(3): 259-263.

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., & Blaxter, M.L. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. *PLoS ONE*. 6(4): e19315.

Blackman, B.K., Michaels, S.D. & Rieseberg, L.H. 2011. Connecting the sun to flowering in sunflower adaptation. *Molecular Ecology*. 20(17): 3503–3512.

Brooks, R.E. & Schofield, E.K. 1986. *Flora of the Great Plains*. Lawrence: University Press of Kansas.

Brown, M.J., Ferruzzi, M.G., Nguyen, M.L., Cooper, D.A., Eldridge, A.L., Schwartz, S.J. & White, W.S. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *The American Journal of Clinical Nutrition*. 80(2): 396-403.

Burke, J.M., Tang, S., Knapp, S.J. & Rieseberg, L.H. 2002. Genetic analysis of sunflower domestication. *Genetics*. 161(3): 1257-1267.

Chapman, M.A. & Burke, J.M. 2012. Evidence of selection on fatty acid biosynthetic genes during the evolution of cultivated sunflower. *Theoretical and Applied Genetics*. 125(5): 897-907.

Chapman, M.A., Pashley, C.H., Wenzler, J., Hvala, J., Tang, S., Knapp, S.J. & Burke, J.M. 2008. A genomic scan for selection reveals candidates for genes involved in the evolution of cultivated sunflower (*Helianthus annuus*). *The Plant Cell Online*. 20(11): 2931-2945.

Clevenger, S. & Heiser, C.B. 1963. *Helianthus laetiflorus* and *Helianthus rigidus*—Hybrids or species? *Rhodora*. 65(762): 121-133.

Craine, J.M. 2006. Competition for nutrients and optimal root allocation. *Plant and Soil*. 285(1-2): 171-185.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., Blaxter, M.L. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*. 12(7): 499–510.

DeHaan, L.R, Van Tassel, D.L. & Cox T.S. 2007. Perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems*. 20(1): 5–14.

Edwards, S.V. 2013. Next-generation QTL mapping: crowdsourcing SNPs, without pedigrees. *Molecular Ecology*. 22(15): 3885-3887.

Ewers, R.M., Didham, R.K., Pearse, W.D., Lefebvre, V., Rosa, I.M.D., Carreiras, J.M.B., Lucas, R.M., & Reuman, D.C. 2013. Using landscape history to predict biodiversity patterns in fragmented landscapes. *Ecology Letters*. (in press).

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to quantitative genetics* (4th edition). Longman Inc., Harlow UK.

Feng, J. & Jan, C.C. 2008. Introgression and molecular tagging of Rf 4, a new male fertility restoration gene from wild sunflower *Helianthus maximiliani* L.*Theoretical and Applied Genetics*. 117(2): 241-249.

Fu, Y.B., & Peterson, G.W. 2012. Developing genomic resources in two Linum species via 454 pyrosequencing and genomic reduction. *Molecular Ecology Resources*. *12*(3): 492-500.

Georgieva-Todorova, J. 1964. Interspecific hybridization in the genus Helianthus. In: *Interspecific hybridization of plants*, Bulgarian Academy of Science, Sofia, Georgieva-Todorova J. (ed), pp 135-82.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S.,Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, K., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Agriculture. Increased food and ecosystem security via perennial grains. *Science*. 328(5986): 1638–1639.

Göksoy, A.T., Demir, A.O., Turan, Z.M. & Da üstü, N. 2004. Responses of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. *Field Crops Research*. 87(2-3): 167–178.

Harlan, J.R. & De Wet, J.M.J. 1977. Pathways of genetic transfer from *Tripsacum* to *Zea mays*. *Proceedings of the National Academy of Sciences*. 74(8): 3494-3497.

Heiser, C.B. 1965. Species crosses in *Helianthus* III. Delimitation of "section". *Annals of the Missouri Botanical Garden*. 52(3): 364-370.

Hulke, B.S. & Wyse, D.L. 2008. Using interspecific hybrids with *H. annuus* L. In: *Proceedings of the 17th International Sunflower Conference*. Cordoba, Spain, pp 729–734.

Jan, C.C. & Ruso, J.A. 2000. Vigor reducing cytoplasms of perennial *Helianthus* species and their nuclear vigor restoration genes in cultivated lines. In: *Proceedings of the 22nd Sunflower Research Workshop*. January 18-19, 2000, Fargo, ND, pp 18-19.

Jan, C.C., Zhang, T.X., Miller, J.F. & Fick, G.N. 2002. Inheritance of rertility restoration for two cytoplasmic male sterility sources of *Helianthus pauciflorus* (rigidus) Nutt. *Crop science*. 42(6): 1873-1875.

Kantar, M., Betts, K., Stupar, B., Hulke, B. & Wyse, D. 2010. The development of perennial sunflower for wildlife and food uses. Poster presented at the 8th Annual Sclerotinia Initiative Meeting, January 20-22, 2010, Bloomington, MN. (Available at www.sunflowernsa.com/research/research-workshop/documents/ Kantar_Perennial_WildlifeFood_10.pdf)

Kantar, M.B., Betts, K., Michno, J.M., Luby, J.J., Morrell, P.L., Hulke, B.S., Stupar, R.M. & Wyse, D.L. 2014. Evaluating an interspecific *Helianthus annuus* x *Helianthus tuberosus* population for use in a perennial sunflower breeding program. *Field Crops Research*. 155:254–264.

Kawakami, T., Morgan, T.J., Nippert, J.B., Ocheltree, T.W., Keith, R., Dhakal, P. & Ungerer, M.C. 2011. Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Molecular Ecology*. 20(11): 318–2328.

Kays, S.J. & Nottingham, S.F. 2007. Biology and chemistry of Jerusalem artichoke*: Helianthus tuberosus L*. CRC press, Baton Rouge, Louisiana.

Kell, D.B. 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Annals of Botany*. *108*(3): 407-418.

Kowalski, R. 2004. Evaluation of chemical composition of some *Silphium* L. species seeds as alternative foodstuff raw materials. *Polish Journal of Food and Nutrition Sciences*. 13(4): 349-354.

Lesica, P. & Allendorf, F.W. 1999. Ecological genetics and the restoration of plant communities: Mix or match? *Restoration Ecology*. 7(1): 42–50

Manel, S., Joost, S., Epperson, B.K., Holderegger, R., Storfer, A., Rosenberg, M.S., Scribner, K.T., Bonin, A., Fortin, M.J. 2010. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*. 19(17): 3760-3772.

Marcel-les-Valence, F. 2005. Pruning intensity and fruit load influence vegetative and fruit growth in an early-maturing peach tree (cv. Alexandra). *Fruits*. 60(2): 133-142.

Muellenborn, C., Krause, J.H. & Cerboncini, C. 2011. Analysis of differential transcript expression reveals time-dependent leaf responses to *Sclerotinia sclerotiorum* in wild and cultivated sunflower. *Plant Molecular Biology Reporter*. 29(3): 597-608.

Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R. & Hohenlohe, P.A. 2013. Genotyping-by-sequencing in ecological and conservation genomics. *Molecular Ecology*. 22(11): 2841–2847.

Otto, S.P. & Lenormand, T. 2002. Resolving the paradox of sex and recombination. *Nature Reviews Genetics.* 3(4): 252-261.

Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S., Manes, Y., Dreisigacker, S., Crossa, J., Sanchez-Villeda, H., Sorrells, M. & Jannik, J.L. 2012. Genomic selection in wheat breeding using genotyping-by-sequencing. *The Plant Genome Journal.* 5(3): 103-113.

Rashid, K.Y. & Seiler, G.J. 2004. Epidemiology and resistance to *Sclerotinia* head rot in wild sunflower species. In *Proceedings of the 16th International Sunflower Conference*. Fargo, ND, August 29-September 4, 2004. pp. 751-754.

Rashid, K.Y. & Seiler, G. 2005, February. Epidemiology and resistance to Sclerotinia head rot in wild sunflower species. In: *Proceedings of the13th International Sclerotinia Workshop*. Monterey, California, USA, 12-16 June, 2005

Ratcliff, W.C., Denison, R.F., Borrello, M. & Travisano, M. 2012. Experimental evolution of multicellularity. *Proceedings of the National Academy of Sciences.* 109(5): 1595-1600.

Reagon, M. & Snow, A.A. 2006. Cultivated *Helianthus annuus* (Asteraceae) volunteers as a genetic "bridge" to weedy sunflower populations in North America. *American Journal of Botany.* 93(1): 127-133.

Rogers, C.E., Thompson, T.E. & Seiler, G.J. 1982. *Sunflower species of the United States*. National Sunflower Association, Bismarck, North Dakota, USA.

Sadras, V.O. & Egli, D.B. 2008. Seed size variation in grain crops: Allometric relationships between rate and duration of seed growth. *Crop Science.* 48(2): 408.

Seiler, G.J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research.* 30(3): 195-230.

Seiler, G.J. & Brothers, M.E. 1999. Oil concentration and fatty acid composition of achenes of *Helianthus* species (Asteraceae) from Canada. *Economic Botany.* 53(3): 273-280.

Sideli, G.M., Schierenbeck, K.A. &Van Tassel, D.L. 2013. Evaluation of interspecific hybrids between wild perennial sunflower and domesticated sunflower for crop use with limited irrigation. Abstract presented at the 24th Graduate Student Meeting of the California Botanical Society, April 14 2013, Berkely California. (Available at www.calbotsoc.org/Symposium_Schedule_files/CentennialProgram_04092013web.pdf).

Siikamäki, P. 1999. Developmental instability in hybrids between *Lychnis viscaria* and *Lychnis alpina* (Caryophyllaceae). *American Journal of Botany.* 86:1683-1686

Sillanpää, M.J. 2011. On statistical methods for estimating heritability in wild populations. *Molecular Ecology.* 20(7): 1324–1332.

Stroup, J.A. Sanderson, M.A., Muir, J.P., McFarland, M.J., & Reed, R.L. 2003. Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. *Bioresource Technology.* 86(1): 65-72.

Timme, R.E., Simpson, B.B. & Linder, C.R. 2007. High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. *American Journal of Botany.* 94(11): 1837-1852.

Travisano, M. & Shaw, R.G. 2013. Lost in the map. *Evolution*. 67(2): 305-314.

van het Hof, K.H., West, C.E., Weststrate, J.A. & Hautvast, J.G. 2000. Dietary factors that affect the bioavailability of carotenoids. *The Journal of Nutrition.* 130(3): 503-506.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications.* 3(5-6): 434-452.

Vear, F. 2011 Helianthus. In: *Wild Crop Relatives: Genomic and Breeding Resources*, Chittaranjan K. (Ed.) Springer, Berlin, pp 161-170.

Velasco, L., Pérez-Vich, B. & Fernández-Martínez, J.M. 2004. Evaluation of wild sunflower species for tocopherol content and composition. *Helia.* 27(40): 107-112.

Vollmann, J. & Rajcan, I. 2010. Oil crop breeding and genetics. In: *Handbook of Plant Breeding* Volume 4, Oil Crops. Johann Vollmann and Istvan Rajcan (Eds). Springer New York, 2010. 1-30.

Weaver, J.E. & Stoddart, L.A. 1935. Response of the prairie to the great drought of 1934. *Ecology*. 16(4): 612-629.

Xia, Y. & Larock, R.C. 2010. Vegetable oil-based polymeric materials: synthesis, properties, and applications. *Green Chemistry*. *12*(11): 1893-1909.

Zimmer, D.E. & Rehder, D. 1976. Rust resistance of wild *Helianthus* species of the North Central United States. *Phytopathology*. 66(2): 208.