An Exciting New Wild Sunflower Species: *Helianthus winteri*

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Abstract

Helianthus winteri, a new wild perennial sunflower species endemic to a small region in central California and formally described in 2013, was collected and accessioned into the National Plant Germplasm System's (NPGS) wild sunflower collection. Seeds are available from the NPGS for research and educational purposes. Oil analyses of wild collected seed indicated a fatty acid profile similar to the mid-oleic NuSun™ hybrids although the high temperature growing conditions for the wild seed probably influenced the fatty acid composition. Analyses of seeds grown under more temperate conditions are planned. Mature *H. winteri* stems become very woody. Preliminary wood chemistry analyses of a lower stem cross-section indicated higher sugar and lower lignin concentrations in *H. winteri* than in cultivated sunflower and two wild annual sunflower species suggesting that a more comprehensive analysis of stem components is warranted. Because genomic analyses indicated a close relationship between *H. winteri* and wild *H. annuus*, transfer of traits from *H. winteri* to improve cultivated sunflower may be a reasonable endeavor.

Introduction

The new, wild perennial sunflower species, *Helianthus winteri*, first reported by Winchell et al. (2012) and described in detail by Stebbins et al. (2013), is endemic to central California with all known populations confined to an area in the western foothills of the Sierra Nevada Mountains in Fresno and Tulare counties between the Kings River and Kaweah River watersheds (Figure 1) southeast of Fresno. Populations have been found on generally south facing, steep, rocky slopes with granitic soils in areas with low or no grazing pressure. To ensure that the sunflower collection in the National Plant Germplasm System (NPGS) contains the maximum possible genetic diversity, we are actively collecting from populations representing the full geographic range of all extant *Helianthus* taxa of this native North American genus. It was of great interest, therefore, to learn about a new species and a high priority to collect seeds from the described populations for the NPGS wild sunflower germplasm collection. For many domesticated crops, germplasm from wild relatives has provided a source of genes for crop improvement leading to disease resistance and tolerance for biotic stresses such as drought and salinity. The sunflower research and breeding community has been particularly successful introgressing useful traits from wild germplasm into cultivated material. *H. winteri* flowers year round and develops woody stems which release notable quantities of a resinous secretion. The secondary vascular growth results in stem persistence after the plants die, giving the appearance of a brush covered hillside in mature populations. Secondary wood production appears to start developing in seedlings as soon as 15 days after germination, visible in stained stem cross-sections (Moyers and Rieseberg 2013), although preliminary genomic data from RNA sequencing of three-week old above ground tissue indicates that *H. winteri* is very similar to wild *H. annuus* (Moyers and Rieseberg 2013). A close genomic relationship between the two species could make transfer of useful characteristics to cultivated sunflower easier than from other perennial wild sunflowers. This paper illustrates the *H. winteri* native area and the species samples in the NPGS wild sunflower collection. We present preliminary data from research directed toward determining if *H. winteri* exhibits traits that could be useful in improving cultivated sunflower.

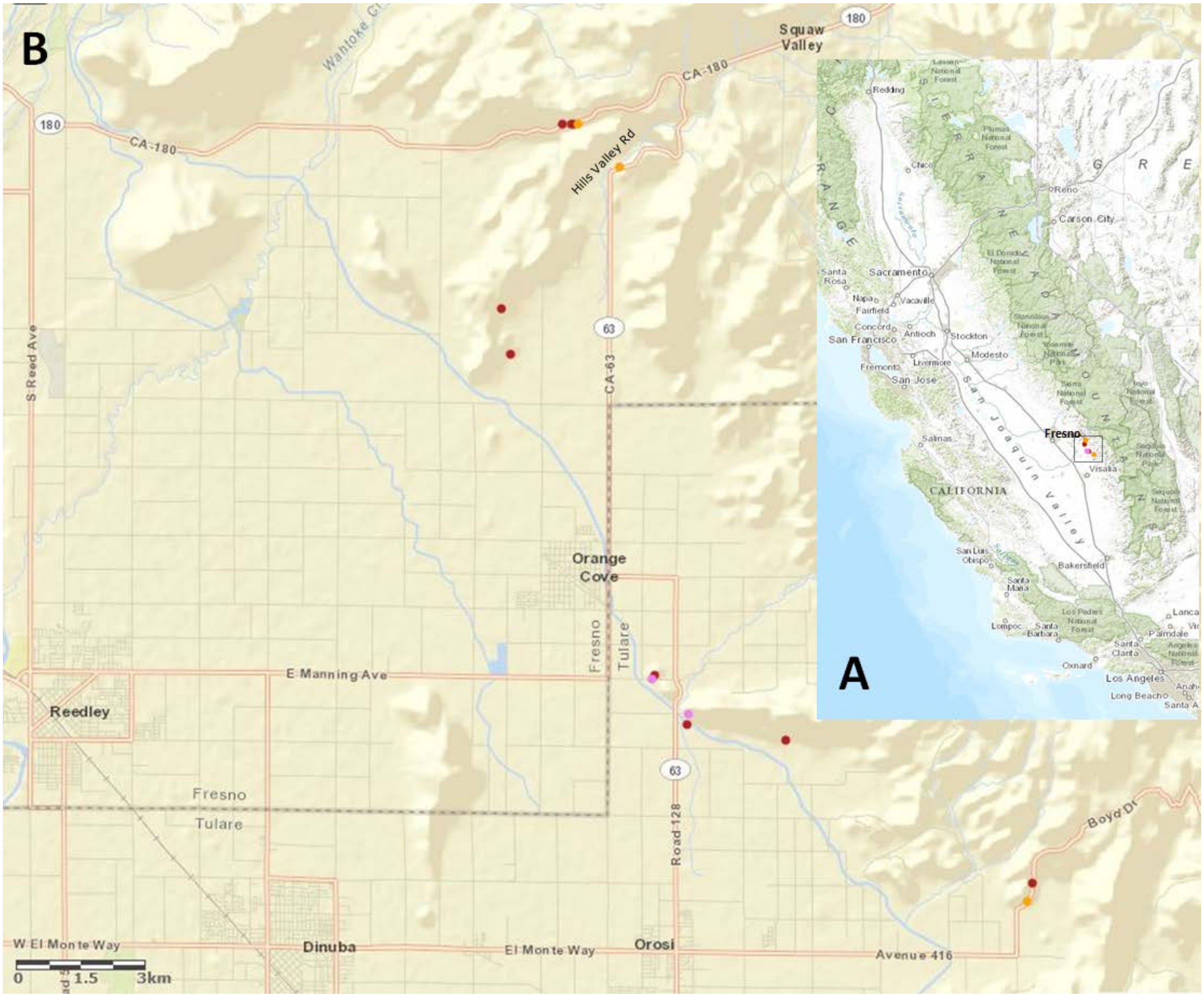

Figure 1. *H. winteri* population locations described by Stebbins et al. (2013,●) (A) Regional presentation of population locations. (B) Within county population locations. Populations sampled by Marek and Seiler (●) and Marek, Larsen, Stebbins and Constable (●) in September 2013 are indicated.

Materials and Methods

Population locations were identified through discussions with local botanists Glenn Cole and John Constable and from the information presented in Stebbins et al. (2013). The Plant Exchange Office (PEO), National Germplasm Resources Laboratory, USDA-ARS in Beltsville, MD supports efforts to expand the national germplasm collections by funding exploration and collection trips. Dr. Marek received funding for a collection trip to California and Oregon for late summer 2013 which included areas within 100 miles of the *H. winteri* populations. The collection trip itinerary was easily modified to include the Sierra Nevada foothills southeast of Fresno and three populations, one of which is not included in the 2013 Stebbins at al. locations, were sampled (Figure 1). In addition, while on a working trip to the USDA-ARS unit in Parlier, CA, Dr. Marek and NCRPIS oilseeds technician Irvine Larsen were able to meet Dr. Stebbins and Dr. Constable and collect from two additional populations.

Seeds were collected from many plants across each population and bulked. Digital images and pressed voucher specimens were taken at each collection site. Voucher specimens were deposited in the USDA-ARS wild *Helianthus* herbarium in Fargo, ND and seed heads were sent to the NCRPIS for processing. The accessions (Table 1) are maintained and distributed through the NCRPIS.

Oil analyses were performed using non-destructive NMR on 6 mL seed samples and fatty acid profiles were determined by gas chromatography on ground and extracted samples of 20 seeds (Granlund and Zimmerman 1975, Metcalfe and Wang 1981, Vick et al. 2004). *H. winteri* analyses were performed in November 2013; remainder of data in Table 2 from cited references.

A *H. winteri* woody stem sample was harvested in the wild in November 2013 by John Constable and shipped to the NCPRIS. *H. argophyllus* stems were harvested from an early June planted field plot at the NCRPIS in September 2013. Stem cross sections were sawed from approximately the same lower portion of stems and sent to the National Renewable Energy Laboratory, Golden, CO for wood chemistry analyses and PCA determinations as described in Ziebell et al. 2013.

ArcGIS on-line mapping software (Esri, Redlands, CA) and Powerpoint were used to construct the map in Figure 1.

Results

Accessions collected:

Each distinct population sampled is considered a separate NPGS accession. Figure 2 illustrates plants and habitat for the population collected along US Hwy 180, east of Fresno. The minimal distance separating populations in the NPGS collections varies by taxon and circumstance. All five of the sampled *H. winteri* populations are accessioned in the NPGS (Table 1). Additional location information and accession availability data can be found in the NPGS Genetic Resources Information Network database (GRIN), http://www.ars-grin.gov/npgs/searchgrin.html.

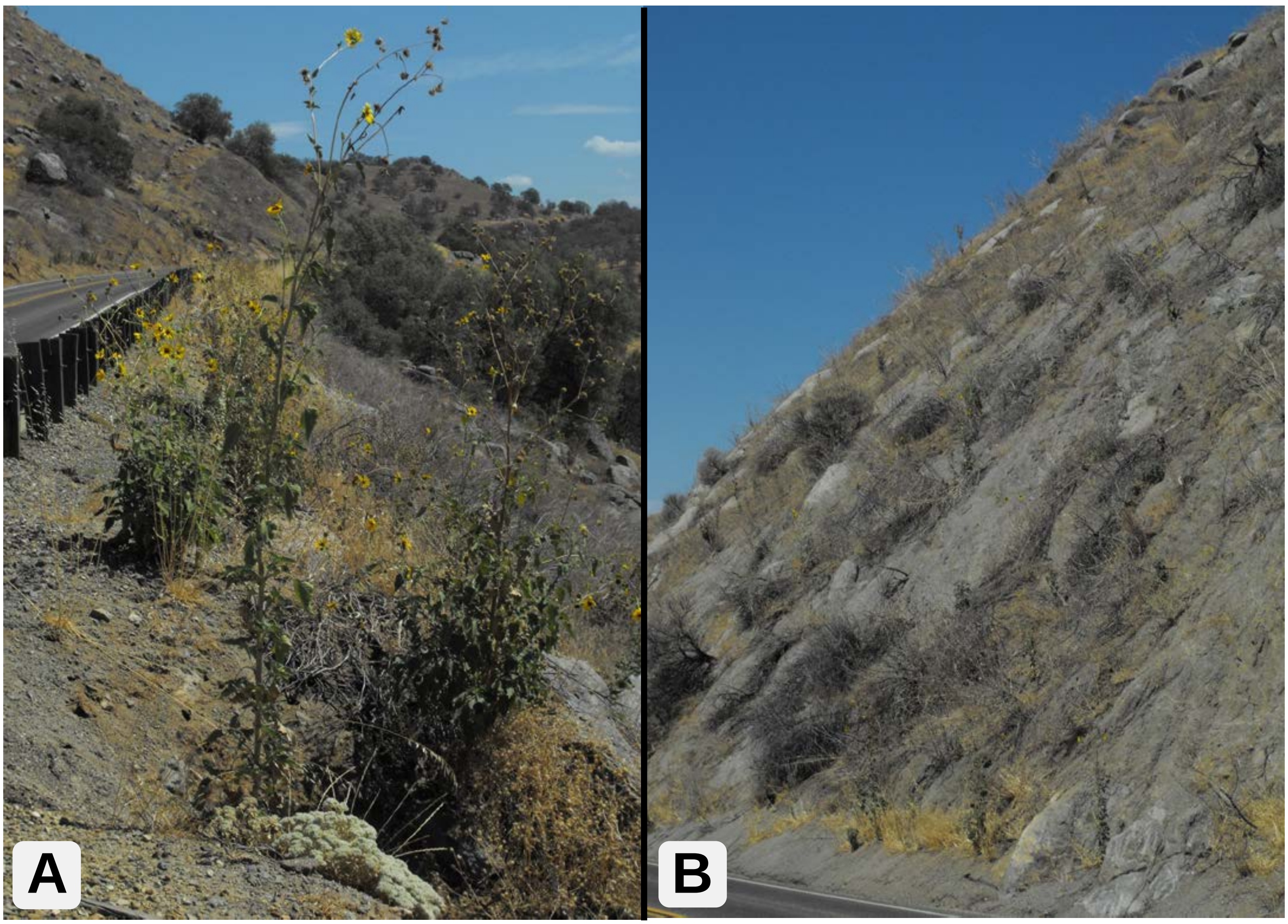

Figure 2. Collection location of Ames 31244. (A) *H. winteri* plants along the south side of Hwy 180, east of Fresno, CA (type collection location). (B) Typical steep, rocky habitat (granitic substrate), living plants and persistent stems of dead plants.

Table 1. Accession numbers and location descriptions, 2013 collected *H. winteri* populations, central California, east of Fresno at the western foothills of the Sierra Nevada Mountains		
Accn number	brief location description	collectors
Ames 32142	both sides of Boyd Rd, northeast of Orosi	Marek, Seiler
Ames 32143	north side of Hills Valley Rd, ~1.5 mi south of Hwy 180	Marek, Seiler
Ames 32144	along Hwy 180 west of junction with Hills Valley Rd	Marek, Seiler
Ames 32152	north side of Ave 448 between Hwy 63 and Hills Valley Rd	Marek, Larsen, Stebbins, Constable
Ames 32153	south slope of Curtis Mt, east of Hwy 63	Marek, Larsen, Stebbins, Constable

Accession analyses:
A trait of primary interest for every sunflower species is seed oil content and quality (fatty acid profile). Only one *H. winteri* collection (Hwy 180 type location) resulted in enough seed to allow oil analyses (Table 2). The *H. winteri* seed samples had higher oil content than samples from two wild annual species, *H. annuus* and *H. argophyllus*, but less oil than cultivated *H. annuus* material bred for high seed oil content. The *H. winteri* seeds had high oleic acid content similar to the levels measured in the mid-oleic cultivated NuSun™ hybrids.

Table 2. Oil analysis data from one *H. winteri* accession compared with samples from two wild annual species and three cultivated lines.

ID	taxon	oil content g kg^{-1}	fatty acid profile, % total oil: palmitic	stearic	oleic	linoleic	arachidic	behenic
Ames 32144	*H. winteri*	250	6.40	2.50	55.90	34.50	0.20	0.30
Ames 32144	*H. winteri*	249	6.30	2.50	52.20	38.00	0.20	0.40
	H. argophyllus	225	7.80	7.30	40.10	44.30	na	na
	wild *H. annuus*	225	5.00	2.70	23.30	68.30	na	na
Hybrid 894	cultivated *H. annuus*	440	7.00	5.00	16.00	70.00	na	na
HA 89	cultivated *H. annuus*	440	6.00	4.10	21.10	69.70	na	na
NuSun™	cultivated *H. annuus*	416	4.43	3.74	62.90	26.56	na	na

H. winteri plants in the wild presented clear morphological differences compared with other sunflower species (Figure 3). Both vegetative young stems and the very woody, mature stems accumulated droplets of a clear resin.

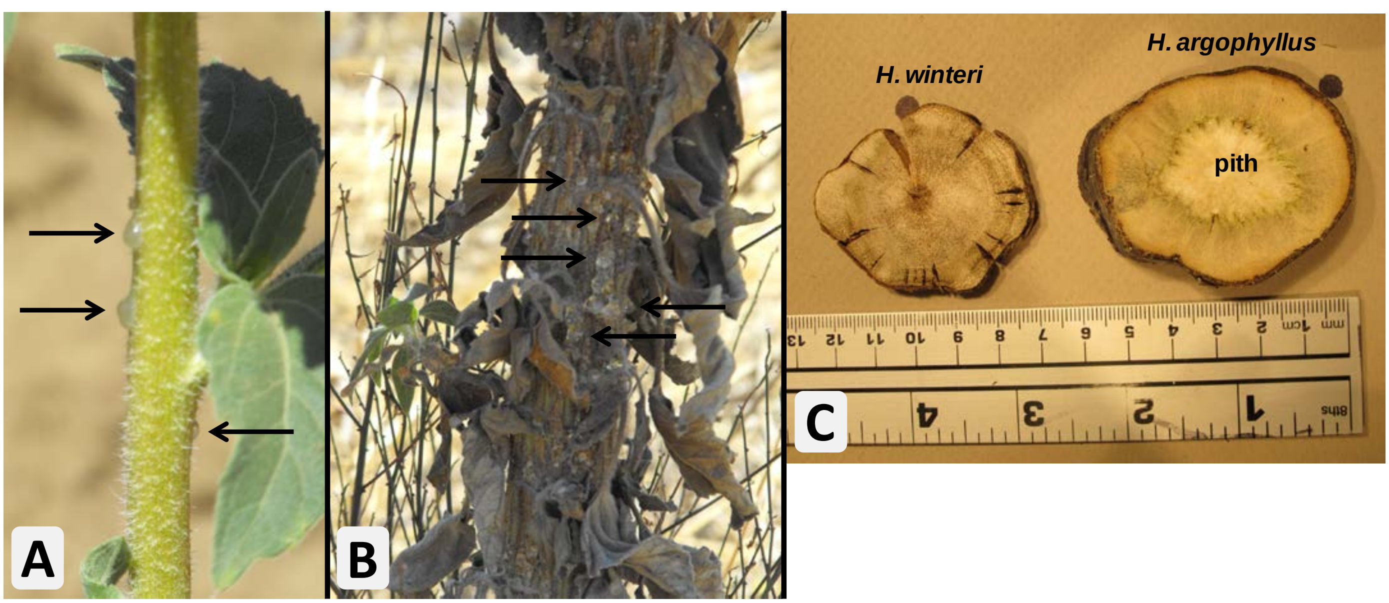

Figure 3. Wild sunflower stems. (A) Young *H. winteri* stem (B) Woody *H. winteri* stem. Arrows point to resin drops. (C) *H. winteri* and *H. argophyllus* stem cross-sections.

Cross sections of the lower portion of an *H. winteri* stem did not have the pith layer commonly observed in annual species such as *H. argophyllus* (Figure 3C).

The *H. winteri* stem cross section used for wood chemistry analysis had a higher proportion of lignin in the syringyl-like (S) fraction than in the guaiacyl-like (G) fraction (Table 3), a desirable characteristic for biomass conversion systems.

Table 3. S/G lignin ratio	
Species	S/G lignin ratio
wild *H. annuus*	1.38
cultivated *H. annuus*	1.50
H. winteri	1.80
H. argophyllus	1.74
Poplar	1.74
Aspen	1.40
Switchgrass	0.44
Miscanthus	0.84
corn stover	1.74
wheat straw	0.94
sorghum	1.10

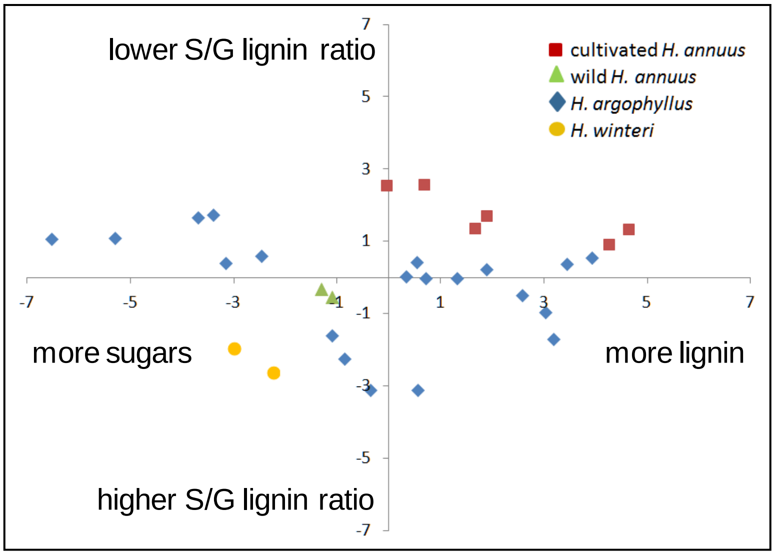

Figure 4. Principal component analyses of wood chemistry data from three sunflower species.

Principal component analyses (PCA) of wood chemistry data separated the *H. winteri* sample from cultivated sunflower and two wild annual sunflower species into the more sugars, less lignin quartile (Figure 4). The wood chemistry data for *H. winteri* are very preliminary because they are derived from a single sample, taken from one location, and sampled in one year. The wild annual species, *H. argophyllus*, has shown a wide range in wood chemistry (Figure 4 data derived from several genotypes sampled at two locations in two years).

Discussion

We successfully located and sampled five populations of the newly described perennial sunflower species, *Helianthus winteri*. The samples are the first accessions of this species in the NPGS wild sunflower collection and add significantly to the diversity in the collection. Samples of the *H. winteri* accessions are available for distribution from the NPGS/NCRPIS for scientific and educational purposes. The species is endemic to a relatively small region in central California delimited by higher elevation mountains directly to the east and land managed for intensive agriculture to the west. In covering such a small geographic area, it is likely that the sampled materials represent limited genetic diversity; therefore, there will be continued exploration of the foothill regions north and south of existing populations in the hope of locating new populations. Oil analysis of two samples of wild collected seed from one *H. winteri* population (Hwy 180 type location) showed the species to have higher oil content than wild *H. annuus* and an oleic acid percentage similar to that bred into the cultivated NuSun™ hybrids. Seed from wild species developed under hot and dry conditions, a likely scenario for the *H. winteri* sample, typically have higher oil concentrations and altered fatty acid profiles from seeds grown under more temperate conditions. It will be of interest to analyze *H. winteri* seed grown under summer agricultural conditions in central Iowa to determine if the concentration and quality characteristics determined for wild collected seed are maintained. *H. winteri* grows in a low rainfall environment – average annual precipitation in the Fresno area is about 11 inches – and there may be drought tolerant traits of value for cultivated sunflower. The stem resin may provide pest resistance, and chemical analyses are planned. The interesting wood chemistry results suggest that it is of value to analyze additional samples and determine more definitively if the *H. winteri* wood contains significantly more sugars and more S lignin units than the genetics currently represented in cultivated sunflower suggesting possible value for development in biomass production. Genomic analyses based on sequencing of RNA isolated from the above ground tissue of three week old seedlings shows a small fixation index difference between *H. winteri* and wild *H. annuus* (Moyers and Rieseberg, 2013) suggesting that transfer of useful traits to cultivated lines will be a reasonable endeavor.

Acknowledgements
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