Gerald J. Seiler* and Laura Fredrick Marek

Collection of Wild *Helianthus anomalus* and *deserticola* Sunflower from the Desert Southwest USA

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Abstract: Genetic resources are the biological basis of global food security. Collection and preservation of wild relatives of important crop species such as sunflower provide the basic foundation to improve and sustain the crop. Acquisition through exploration is the initial step in the germplasm conservation process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America. An exploration covering 3,700 km to the desert southwest United States in mid-June of 2015 led to the collection of five populations of *H. deserticola* (desert sunflower) and 10 *H. anomalus* (sand sunflower) accessions. All populations were collected throughout the broad distributional range of the species. Based on sand sunflower's occurrence in desert sand dune habitats of Utah and Arizona, it frequently has been recognized as drought tolerant, with the largest achenes of any wild species and relatively high oil concentration potential, and thus is a candidate for improving cultivated sunflower. Desert sunflower is a xerophytic annual species found in sandy soils underlain with clay soils on the floor of the Great Basin Desert in small populations in western Nevada, west central Utah, and along the border of Utah and Arizona. Population size, habitat, soil type, seed set, the presence of diseases and insects, and other wild sunflower species located near the collection sites were recorded for each population. This germplasm will be important now and in the future as a genetic resource for the global sunflower crop and at the same time conserve it for future generations.

Keywords: sunflower, crop wild relatives, wild species, germplasm resources, exploration

***Corresponding author: Gerald J. Seiler,** Sunflower and Plant Biology Research Unit, USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58102–2765, USA, E-mail: gerald.seiler@ars.usda.gov
Laura Fredrick Marek, Iowa State University and USDA-ARS, NCR Plant Introduction Station, National Plant Germplasm System, Ames, IA 50014, USA, E-mail: lmarek@iastate.edu

Introduction

Crop wild relatives (CWR), which include the progenitors of crops as well as other species more or less closely related to them, have been undeniably beneficial to sustaining modern agriculture, providing plant breeders with an extensive pool of potentially useful genetic resources dating back 100 years (Hajjar and Hodgkin, 2007). Collection and preservation of CWR from important crop species such as sunflower provide the basic foundation to advance and sustain the crop. Genetic resources are the biological basis of global food security, and acquisition through exploration is the initial step in the germplasm conservation and utilization process. There are 53 species of wild *Helianthus* (39 perennial and 14 annual) native to North America (Heiser *et al.*, 1969; Schilling, 2006). The narrow genetic base of cultivated sunflower has been broadened by an infusion of genes from the wild species, which have provided a continuous source of agronomic and economic traits for cultivated sunflower (Seiler and Rieseberg, 1997; Seiler and Marek, 2011; Kane *et al.*, 2013; Seiler and Jan, 2014; Marek, 2016; Seiler and Marek, 2016).

Hajjar and Hodgkin (2007) surveyed the introduction of genes from CWR in 13 cultivated crops of major importance to global food security from the mid-1980s to 2005. The number of CWR contributed traits for sunflower was seven, mainly for pest and disease resistance, abiotic factors, and male fertility or cytoplasmic male sterility, fifth among the crops surveyed.

The need for germplasm exploration is driven by the priority needs of the crop for new traits. Castañeda-Alvárez *et al.* (2016) assessed the collection priority of sunflower CWR as medium priority because of the long history of CWR use in crop improvement that has benefited from a relatively extensive germplasm collection. However, there are still taxa lacking comprehensive *ex situ* conservation resulting in gaps in the sunflower collection. Kantar *et al.* (2015) used occurrence, bioclimatic, and biophysical data for 36 taxa of sunflower CWR to predict species hotspots and species gaps. This generated a gap analysis that sets the priority needs for further collection of each species and identification of possible sources of abiotic stress traits for plant breeding programs. Twenty-six CWR of sunflower were rated, with 10 high and 16 medium priorities for collection. Interestingly, *H. anomalus* S. F. Blake was identified as a target species, particularly for abiotic stress tolerance and adaptation to extreme soil properties (Kantar *et al.*, 2015).

Helianthus anomalus (sand sunflower) is a rare endemic species adapted to sand dune and swale habitats in Utah and northern Arizona (Heiser, 1958; Heiser *et al.*, 1969; Thompson *et al.*, 1981; Nabhan and Reichhardt, 1983) (Figure 1(a)). It is

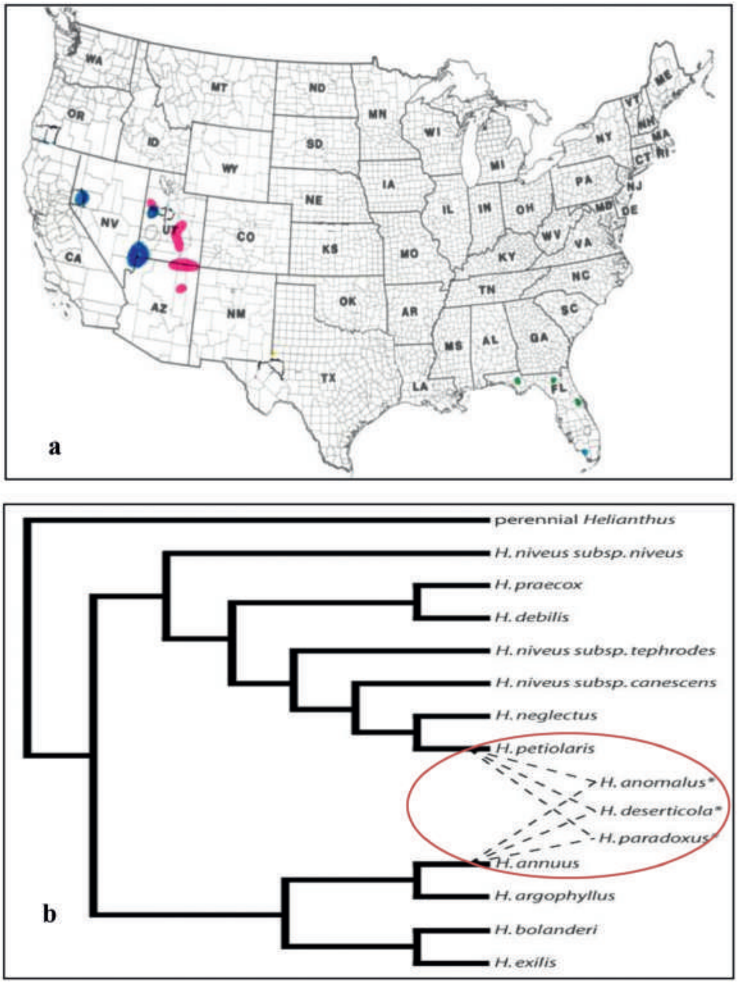

Figure 1: Distribution of *H. anomalus* and *H. deserticola* in the southwest United States and the evolutionary relationships among annual *Helianthus* species. (a) Distribution of *Helianthus anomalus* (sand sunflower ●) in Utah and Arizona, and *H. deserticola* (desert sunflower ●) in Nevada, Utah and Arizona in the desert southwest US. (b) Homoploid hybrid species *H. anomalus* and *H. deserticola* are indicated with asterisks in the red circle. Figure is redrawn from Gross and Rieseberg (2005), and based on combined nuclear ribosomal and chloroplast DNA data reported by Rieseberg (1991).

a confirmed homoploid diploid hybrid species based on comparison of isozyme, nuclear ribosomal DNA, and cpDNA with its parental species, *H. annuus* L. and *H. petiolaris* Nutt. which occupies a more extreme environment than either parental species (Rieseberg, 1991; Gross *et al.*, 2004; Ludwig *et al.*, 2004) (Figure 1(b)). *Helianthus annuus* is distributed throughout the central and western United States and typically inhabits heavy, clay-based soils. *Helianthus petiolaris*, the smaller of the two parental species, is distributed mainly through the central United States and inhabits sandier soils than *H. annuus*. The two parental species co-occur and often hybridize throughout their range. The species are all annual, outcrossing, and have a haploid chromosome number of n = 17 (Heiser, 1947; Heiser *et al.*, 1969; Rogers *et al.*, 1982). *Helianthus anomalus* has been frequently recognized as drought tolerant, with the largest achenes of any wild species and relatively high oil concentration potential (Seiler, 2007), and thus is a candidate for improving cultivated sunflower (Nabhan and Reichhardt, 1983; Seiler *et al.*, 2006). It also appears to be more tolerant of nutrient stress than its ancestral parents, based on its slower relative growth rate and higher nutrient-use efficiency (Brouillette and Donovan, 2011).

Helianthus deserticola Heiser (desert sunflower) is a xerophytic species found in sandy soils of the Great Basin Desert underlain with clay and distributed in small populations located in western Nevada, west central Utah, and along the border of Utah and Arizona, USA (Heiser *et al.*, 1969) (Figure 1(a)). It is also a homoploid diploid annual hybrid between two annual parental diploid species, *H. annuus* and *H. petiolaris* (Rieseberg, 1991; Gross *et al.*, 2004). This species inhabits the desert, an extreme environment relative to its diploid parental species (Gross *et al.*, 2004). Based on the occurrence of desert sunflower in sand dune desert habitats, it frequently has been recognized as drought tolerant with a relatively high oil concentration potential, and thus is a candidate for improving cultivated sunflower (Seiler, 1992, 2007). Both *H. anomalus* and *H. deserticola* are excellent candidates for diversifying the genetic base of cultivated sunflower by enhancing oil concentration and quality improvement, as well as drought tolerance.

Unfortunately, very few populations of *H. anomalus* and *H. deserticola* have been collected and only a few are available from the U.S. Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (NPGS) wild sunflower germplasm collection (Marek, 2016). It is also very difficult to regenerate the limited number of original seeds from some of the earlier collected accessions. The objectives of this study were: 1) undertake an exploration to the desert southwest USA in Utah and Arizona to collect the winter-spring populations in June instead of the summer-fall populations previously collected in September-October of the two desert species, *H. anomalus* and *H. deserticola*; and 2) preserve them for future generations to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable and competitive global crop.

Materials and methods

The sunflower exploration for *H. anomalus* and *H. deserticola* took place from June 14 to June 22, 2015. Some populations were revisited in late July-early August, 2015 to collect additional seed, and two additional populations without mature seed in June were collected. The exploration covered 3,700 km in two states, Utah and Arizona. Seed heads were collected from 20 to 250 plants within each population and bulked into a single sample. Herbarium specimens were deposited in the USDA-ARS wild *Helianthus* herbarium at Fargo, ND. The achene samples were deposited at the USDA-ARS North Central Regional Plant Introduction Station, Ames, Iowa, where they are maintained and distributed.

All populations were collected from the restricted distributional range of the species: Utah for *H. anomalus*, and Utah and Arizona for *H. deserticola* (Figure 1(a)). Prior locations, generalized distribution maps, and herbaria voucher records were used to locate populations in cooperation with local natural resource officials and botanists who provided valuable information about the current year population distributions and status of the two species. Land ownership was determined and all necessary permits and permissions were obtained for seed collection and inclusion of the seed in the USDA-ARS-NPGS gene bank. Population size (number and extent), habitat, soil type, seed set per head, and the presence of diseases, insects, and other wild sunflower species were recorded for each population.

Results and discussion

The location of the 10 *H. anomalus* populations in Utah and five *H. deserticola* collected in Utah and Arizona are shown in Figure 2. Table 1 contains the location where the populations were collected, detailed habitat description, elevation, and the general population size. A single population of *H. anomalus* was observed in Arizona, but the plants were just flowering and no seeds were available. It had been 15 years since this species was last collected for the NPGS (Seiler and Brothers, 2003). Attempts to recollect this endemic species over the last quarter century have met with mixed results. In September of 2000, none of 12 populations collected in October of 1980 could be relocated in the fragile sandy habitats (Seiler and Brothers, 2003). The species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during mid-June located numerous populations of sand

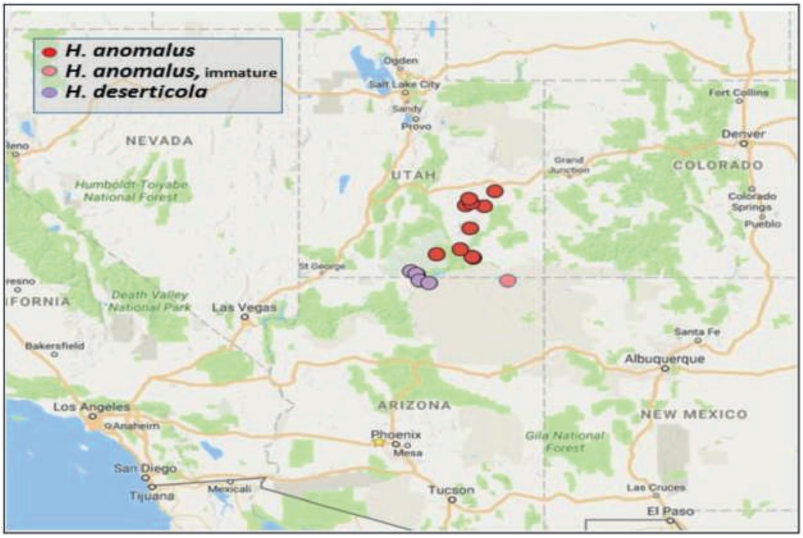

Figure 2: Map showing the locations of the 10 *H. anomalus* populations in Utah and five *H. deserticola* collected in Utah and Arizona collected during the exploration in mid-June-early August, 2015.

Table 1: *Helianthus anomalus* and *H. deserticola* identification number, elevation, location, habitat, and population size collected in June, 2015.

Identification Number	Species	Elevation (m)	Location	Habitat	Population Size
Ames 32938	*H. anomalus*	1310	**Utah**; San Juan Co., SE of Cal Black Memorial Airport	Shifting sand dunes, roadside	200
Ames 32939	*H. anomalus*	1450	**Utah**; San Juan Co., Nokai Dome Rd, SE of Halls Crossing	Shifting sand dunes, roadside	750
Ames 32941	*H. anomalus*	1147	**Utah**; Garfield Co., Notum-Bullfrog Rd	Shifting sand dunes, roadside	1,000
Ames 32943	*H. anomalus*	1769	**Utah**; Kane Co., Hole-in-the-Rock Rd, Grand Staircase Escalante National Monument	Shifting sand dunes, roadside	200
Ames 32945	*H. anomalus*	1394	**Utah**; Garfield Co., unnamed draw into North Wash, west side of Hwy 95	Shifting sand dunes, steep slope	250

(*continued*)

Table 1: (*continued*)

Identification Number	Species	Elevation (m)	Location	Habitat	Population Size
Ames 32946	*H. anomalus*	1425	**Utah**; Wayne Co., near Hanksville	Shifting sand dunes, roadside	200
Ames 32947	*H. anomalus*	1661	**Utah**; Wayne Co., Lower San Rafael Rd	Shifting sand dunes	100
Ames 32948	*H. anomalus*	1565	**Utah**; Emery Co., Hans Flat Rd	Shifting sand dunes	1,000
Ames 32949	*H. anomalus*	1231	**Utah**; Grand Co., White Wash Dunes	Shifting sand dunes	1,000
Ames 32950	*H. anomalus*	1532	**Utah**; Emery Co., west side of Hwy 24	Shifting sand dunes	1,000s
Ames 32930	*H. deserticola*	1214	**Utah**; Kane Co., beside High Desert Lodge, Big Water	Sandy desert shrub pasture	750
Ames 32931	*H. deserticola*	1290	**Utah**; Kane Co., end of Church Wells Rd, Grand Staircase Escalante Natl. Monument	Sand dunes, near pasture	500
Ames 32933	*H. deserticola*	1261	**Utah**; Kane Co., Jacobs Tank Rd, west of Grand Staircase Escalante National Monument visitor center	Sandy swale	500
Ames 32934	*H. deserticola*	1294	**Arizona**; Coconino Co., Vermilion Cliffs Natl. Monument; Ferry Swale Wash	Undulating swale wash, sandy soil	1,000
Ames 32935	*H. deserticola*	1295	**Arizona**; Coconino Co., SE of Page	Sandy roadside ditch	1,000

sunflower, probably due to the much above normal 2015 spring rains in several parts of the species' distributional range.

The exploration also successfully collected five representative populations of *H. deserticola* from its distributional range in Utah and Arizona (Figure 2). *Helianthus deserticola* was not collected in Nevada because of restricted access to the areas where the species occurs. In September of 2000, an attempt to locate several populations previously collected in October of 1980 was not successful in the fragile sandy sagebrush habitat, probably due to the extremely dry 2000 growing season, however, one new population was discovered (Seiler and Brothers, 2003). As with *H. anomalus*, the species appears to be very sensitive to the prevailing fall-winter and spring-summer moisture conditions. The current exploration during June 2015 located several populations of desert sunflower mainly due to the much above normal spring rains in several parts of the species' distributional range.

Typical habitat of the only population of *H. anomalus* located in Arizona is shown in Figure 3. Unfortunately, only a few plants were observed with no mature seeds to collect. Figure 4 shows one of the diverse habitats in Utah where typical *H. anomalus* (Ames 32938) plants grow on top of sandy hummocks with the wind causing the sand to shift and appear as waves in the sand. A typical plant of *H. anomalus* (Ames 32939) in Utah with multiple branches and heads, shiny light green leaves, and whitish stems can be seen in Figure 5. In Figure 6, *H. anomalus* (Ames 32941) plants illustrate the unique tap roots that develop to help plants survive the constant shifting sand on the dunes in Utah. A unique habitat for *H. anomalus* (Ames 32945) is in a draw on the steep slope of a shifting sand dune in Utah seen in Figure 7. Dried white plant stalks from the previous

Figure 3: Gerald Seiler standing next to the largest *H. anomalus* plant in a very small population found near Dennehosto, Arizona in a shifting sand dune. Only a few plants were found with no mature seed to collect.

Figure 4: *Helianthus anomalus* (Ames 32938) on hummock sand dunes in San Juan County, Utah, southeast of Cal Black Memorial Airport. Notice the wave pattern in the sand from the wind shifting the sand in the dunes.

Figure 5: Population of *H. anomalus* (Ames 32939) in San Juan County, Utah, southeast of Halls Crossing in shifting sand dunes. Typical plants with multiple branches, shiny light green leaves, and whitish stems.

Figure 6: *Helianthus anomalus* (Ames 32941) population in Garfield County, Utah, along Notum-Bullfrog Rd in shifting sand dunes near roadside. Notice the distorted exposed roots that developed to anchor the plant in the actively shifting sand dunes.

Figure 7: Population of *H. anomalus* (Ames 32945) in Garfield County, Utah, North Wash, west side of Hwy 95, with sunflowers growing in a draw of a steep slope of a shifting sand dune.

season(s) confirm a persistent and thriving population of sand sunflower (Ames 32941) in Utah as seen in Figure 8.

While *Helianthus deserticola* shares some of the habitat types of *H. anomalus*, however, the main difference is that it is found on the floor of the Great Basin Desert in sandy soils underlain with clay interspersed mainly with sagebrush and other desert shrubs. The typical desert shrub habitat of a population of *H. deserticola* (Ames 32930) located in southern Utah is shown in Figure 9. Figure 10 shows one of the diverse habitats where *H. deserticola* (Ames 32931) grows in sandy soils and hummock type of topography near desert shrubs in Utah. Typical *H. deserticola* (Ames 32933) plants in Utah with multiple branches and heads, dull green leaves and darker greenish-red pubescent lower stems can be seen in Figure 11. Figure 12 shows *H. deserticola* (Ames 32934) plants distributed in a unique habitat in an undulating swale wash in sandy soil among the desert shrubs in Arizona. A unique habitat of *H. deserticola* (Ames 32934) plants in Arizona scattered in a sandy pocket on an undulating swale wash underlain by shale rock (Figure 13). Figure 14 shows Ames 32935 *H. deserticola* plants in a sandy roadside ditch with rather thick desert shrubs typical of the desert sunflower in Arizona.

Figure 8: Laura Marek collecting seed of *H. anomalus* (Ames 32941) in Garfield County, Utah, along Notum-Bullfrog Rd, in shifting sand dunes. Note the dead white plant stalks from previous season(s).

Figure 9: *Helianthus deserticola* (Ames 32930) in Kane Co., Utah, near Big Water in a typical desert shrub habitat interspersed among the shrubs in open sandy areas.

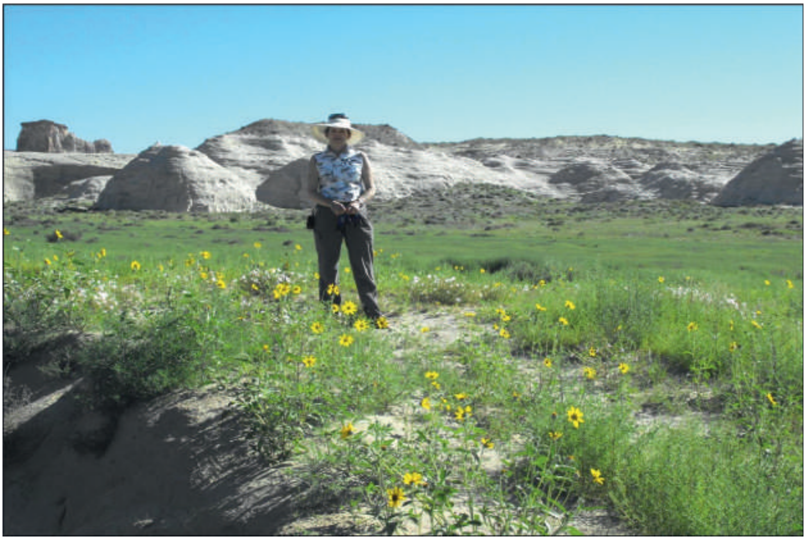

Figure 10: Laura Marek collecting seed of *H. deserticola* population (Ames 32931) in Kane Co., Utah, at the end of Church Wells road, Grand Staircase Escalante National Monument. Note the different habitat with more grayish sandy soils and hummock type topography in the background near desert shrubs.

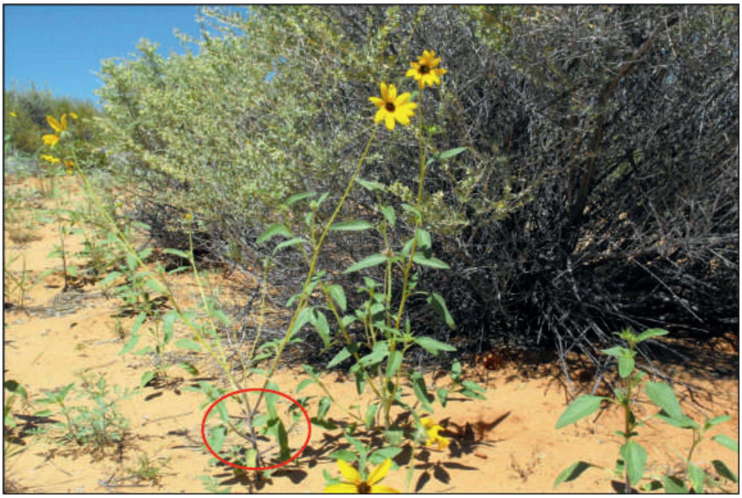

Figure 11: *Helianthus deserticola* (Ames 32933) population in Kane Co., Utah, along Jacobs Tank Rd, west of the Grand Staircase Escalante National Monument visitor center, sandy pocket among the desert shrubs. Typical plant with multiple branches, dull green leaves, and darker greenish-red pubescent lower stems (red circle).

Figure 12: Population of *H. deserticola* (Ames 32934) in Coconino Co., Arizona, Vermillion Cliffs National Monument, Ferry Swale Wash, in an undulating swale wash in sandy soil among the desert shrubs.

Figure 13: Gerald Seiler collecting seed of *H. deserticola* (Ames 32934) in Coconino Co., Arizona, Vermillion Cliffs National Monument, Ferry Swale Wash, in sandy soil in the undulating swale wash among the desert shrubs. Note the white shale outcropping in the background that underlies this area (red arrow).

Figure 14: *Helianthus deserticola* (Ames 32935) population in Coconino Co., Arizona, in a sandy roadside ditch in thick desert shrubs, east of Page.

Wild species crop relatives of cultivated sunflower such as *H. anomalus* and *H. deserticola*, native to drought-prone environments, are potential sources of drought resistant traits for improving crop productivity under water-limiting conditions. Collection of this germplasm provides the opportunity to study in more detail the physiological processes that are involved in the survival mechanisms of these desert-inhabiting species, similar to the study by Bowsher *et al.* (2016) for desert-adapted *Helianthus niveus* (Benth.) Brandegee ssp. *tephrodes* (A. Gray) Heiser endemic to the Algodones Dunes in California, part of the Sonoran desert.

The populations of *H. anomalus* and *H. deserticola* described above, as well as other accessions of wild species can be obtain from the USDA-ARS-NPGS wild sunflower species gene bank collection through the GRIN Global system that serves as the portal for requesting germplasm using the following link: (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). One will have to register as a web cooperator and set up an order to request seeds. The germplasm is freely available for research and educational purposes, although some restrictions are imposed by import regulations of receiving countries.

Conclusion

The addition of 10 *H. anomalus* and five *H. deserticola* populations to the NPGS wild sunflower germplasm collection represents the first germplasm of these species collected from Utah and Arizona in almost 15 years. Conservation of this genetic resource is important since emerging diseases of plants and other agricultural pests are predicted to become more common and damaging in a warming climate with environmental uncertainty in the future (Anderson *et al.*, 2004; Tyack and Dempewolf, 2015). Historically, crop wild relatives of sunflower have played a vital role in the development of a viable sunflower crop and will continue to do so as one of the predominant sources of genetic diversity for the sunflower crop. Diverse wild crop relatives of sunflower will enable the crop to remain a viable and competitive global crop and at the same time these genetic resources are conserved and available for future generations.

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