

2008 update: The USDA sunflower collection at the north central regional plant introduction station, Ames, IA, USA

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ABSTRACT

An update on the status of the *Helianthus* L. germplasm collection in the National Plant Germplasm System of the United States is presented. The collection is held at the North Central Regional Plant Introduction Station in Ames, IA administered by the USDA-ARS Plant Introduction Research Unit in cooperation with the Agricultural Experiment Station at Iowa State University. Sunflower germplasm is acquired, maintained, characterized and distributed as well as used to conduct and support germplasm-related research. Overall, the *Helianthus* collection is 86% available and is composed of accessions of the domesticated species *Helianthus annuus* and its wild relative taxa. Sunflower germplasm and associated information are freely available to scientists and educators world-wide for research, crop improvement and product development.

Key words: cultivated – germplasm collection – *Helianthus* – wild.

INTRODUCTION

The sunflower (*Helianthus* L.) germplasm collection in the National Plant Germplasm System (NPGS) of the United States is held at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA. The collection contains accessions for all but one of the 63 taxa recognized in the recent Flora of North America (FNA) treatment of *Helianthus* (Schilling, 2006) as well as accessions of *Helianthus niveus* ssp *canescens* and *Helianthus* x *multiflorus* (neither are considered in the Flora at this time). Forty-two of the taxa are perennial and 21 are considered annual, including the cultivated species, *Helianthus annuus*. The genus *Helianthus* originated in North America and is well distributed across the continent although a number of *Helianthus* taxa have restricted ranges. Two species are on the U.S. Fish and Wildlife Threatened and Endangered Species List. This article describes the sunflower collection at the NCRPIS and briefly outlines some of the recent research we have conducted using this germplasm.

MATERIALS AND METHODS

In 2007, seed was received for 96 wild sunflower accessions collected during two NPGS Plant Exchange Office (PEO) sponsored field trips by Gerald Seiler and Thomas Gulya (USDA Sunflower Research Unit, Fargo, ND) in Australia and by Gerald Seiler and Laura Fredrick Marek (NCRPIS, Ames, IA) in the southwestern U.S. Since the previously published collection summary (Marek et al., 2004), five PEO sponsored collection trips and four independently funded forays have resulted in the addition of 288 accessions of wild populations to the collection. Thirty-one cultivated accessions have been received in the active collection, primarily expired Crop Science Registry (CSR) materials.

We continue to experiment with methods to improve germination of wild sunflower accessions and recently modified our protocol so that seeds are rinsed in cool, running tap water for seven days instead of 24 hours before transfer to germination paper (wild *H. annuus*) or germination boxes (all other wild taxa). Seeds are incubated for up to 8 weeks at 4 °C before being moved to germinators (20/30 °C, 12/12 hr light/dark cycles or 15/25 °C, 14/10 hr light/dark cycles). Seedlings are transplanted to flats and established in the greenhouse before being transplanted to the field. Wild accessions are caged and screened before flowering to maintain the genetic integrity of each accession and honey bees are introduced into the cages for pollination. Increase plantings of cultivated *H. annuus* accessions are direct seeded except accessions with low quantity or low quality seed are started in incubators, transplanted in the greenhouse, and moved to the field as for wild sunflower accessions. Most cultivated accessions are hand pollinated after bagging the primary inflorescence and efforts are made during hand pollination to

mix the pollen within each population. In 2007, several cultivated accessions with significant branching were successfully increased in cages using insect pollinators. After harvesting, drying and cleaning, seeds are stored at 4C and 35% humidity.

A number of wild taxa require a longer growing season than reliably occurs in central Iowa. In 2004, we began a significant partnership with the NPGS Parlier, CA location in the San Joaquin Valley (USDA-ARS National Arid Land Plant Genetic Resource Unit, NALPGRU). Average first frost at this location occurs in early December; average first frost in Ames, IA occurs during the first week in October. Seedlings are shipped to Parlier where they are transplanted and managed through harvest, including caging and use of introduced pollinators. Harvested material is shipped to Ames for processing.

Plasmopara halstedii (Farlow) Berl and de Toni is the causal agent of downy mildew and is a major phytosanitary issue for seed exported from the United States. A two-part disease management plan is employed to ensure production of *Plasmopara halstedii*-free seed (Marek et al., 2004). First, seeds intended for direct-seeding in the field are treated with Allegiance (metalaxyl) fungicide. Secondly, all plants in the field are visually inspected for systemic downy mildew infection a minimum of two times before flowering, initially at the V6-V8 growth stage. It is relatively easy to inspect every plant because seed production fields at the NCRPIS are not large. Infected plants are rare, but if encountered are physically removed from the field. Follow-up inspections are done at 4-7 day intervals until it is apparent that no infected plants are present. Plants are also inspected for symptoms of bacterial and viral diseases. Field inspections have been routinely conducted since 1990. Seed lots produced before 1990 are treated with metalaxyl fungicide prior to overseas shipment if the destination country accepts chemically-treated seed.

RESULTS

The NCRPIS collection currently contains 3,838 accessions representing 64 *Helianthus* taxa (Tables 1 and 2). The only sunflower taxa not present in the collection are *H. nuttallii* ssp *parishii* (likely extinct) and *H. niveus* ssp *niveus* (endemic to Baja California, Mexico and to our knowledge not currently available for distribution by non-Mexican genebanks). The largest proportion of the collection is made up of wild (24%) and cultivated (44%) *Helianthus annuus* accessions of which 94% are available for distribution. Non-*Helianthus annuus* wild annual accessions account for 12% of the collection and are 86% available. Perennial wild accessions, representing 20% of the collection, are 59% available. This information is summarized in Table 3. Approximately the same percentage of *H. annuus* accessions is available as reported in 2004, the non-*annuus* wild annual accession availability has increased 15%, and availability of the wild perennial accessions has more than tripled from the 18% available four years ago to 59% currently. Accessions are available for 25 taxa which had no distributable germplasm in 2004 (Tables 1 and 2). The absolute number of accessions in the sunflower collection reflects a balance between acquisitions from collections and donations and inactivations. Nearly 350 non-viable sunflower accessions have been inactivated since 2004, primarily accessions collected prior to 1980 which did not germinate during one to several increase attempts. The percentage of available germplasm increases as a result of successful production in Ames and in Parlier.

The partnership with the NALPGRU staff at Parlier has resulted in increased availability of a number of taxa including *H. agrestis*, *H. argophyllus*, *H. exilis*, and *H. radula*. The *H. argophyllus* collection is expected to be fully available after the 2008 growing season. To complement the versatility that Parlier provides, protocols have been tested in Ames to manipulate daylength and induce earlier flowering in selected taxa (Marek 2008). In 2007, black landscape fabric was used within the confines of a 20 x 50 screened hoop house to increase the dark period for an accession of *H. argophyllus*. From mid-July until mid-August plants were covered daily, increasing the dark period from about 9 hours to 16 hours. As a result, treated plants were flowering at the time treatment ended; control plants did not begin to flower for six more weeks and only covered plants produced significant seed quantities before the Ames area received its first killing frost. This protocol will be tested during 2008 with additional late flowering taxa.

Table 1. Annual *Helianthus* taxa in the NCRPIS collection. Taxa unavailable in 2004 are shown in boldface type.

Annual taxa	# accns	# avail accns
<i>H. agrestis</i>	5	2
<i>H. annuus</i> , cultivated	1660	1584
<i>H. annuus</i> , cultivated, CSR*	42	0
<i>H. annuus</i> , wild	931	894
<i>H. anomalus</i>	8	4
<i>H. argophyllus</i>	44	30
<i>H. bolanderi</i>	8	4
<i>H. debilis</i> ssp <i>cucumerifolius</i>	11	11
<i>H. debilis</i> ssp <i>debilis</i>	13	11
<i>H. debilis</i> ssp <i>silvestris</i>	22	22
<i>H. debilis</i> ssp <i>tardiflorus</i>	5	4
<i>H. debilis</i> ssp <i>vestitus</i>	3	3
<i>H. deserticola</i>	22	15
<i>H. exilis</i>	31	25
<i>H. neglectus</i>	28	28
<i>H. niveus</i>	1	1
<i>H. niveus</i> ssp <i>canescens</i>	19	13
<i>H. niveus</i> ssp <i>tephrodes</i>	12	6
<i>H. paradoxus</i> **	10	0
<i>H. petiolaris</i>	15	13
<i>H. petiolaris</i> ssp <i>fallax</i>	30	30
<i>H. petiolaris</i> ssp <i>petiolaris</i>	94	94
<i>H. porteri</i>	8	8
<i>H. praecox</i>	2	2
<i>H. praecox</i> ssp <i>hirtus</i>	7	7
<i>H. praecox</i> ssp <i>praecox</i>	8	8
<i>H. praecox</i> ssp <i>runyonii</i>	24	24
<i>H. sp.</i>	11	10
<i>H. hybrid</i>	14	13
total	3088	2866

*CSR: accessions covered by Crop Science Registry protection. Distributable after protection expires or with the authorization of the inventor.

**Species covered by the U.S. Fish and Wildlife Threatened and Endangered Species Act

Morphological and phenological observations are collected during the growing season. Descriptor information is also contributed by collaborators at the USDA Sunflower Research Group in Fargo, ND. We travel to Parlier annually to record descriptor data for accessions increased at the NALPGRU. Information is entered into the Germplasm Resources Information Network (GRIN) database maintained by the USDA-ARS Database Management Unit of the NPGS GRIN can be accessed at <http://www.ars-grin.gov/npgs/searchgrin.html>. Descriptor information is available through the 'Research Crops and Descriptor/Evaluation Data Queries' option. Select "SUNFLOWER"; select "List of Descriptors." Descriptor information can also be viewed under "OBSERVATIONS" when querying GRIN for specific accessions under "Accession Area Queries" at the "search grin" url given above. During 2007 we began loading flower, plant and seed images to GRIN for all accessions increased in Ames and Parlier. "Image" is considered an "uncharacterized descriptor" on the sunflower descriptor page in GRIN and can also be accessed under "OBSERVATIONS" when viewing specific accessions.

Table 2. Perennial *Helianthus* taxa in the NCRPIS collection. Taxa unavailable in 2004 are shown in boldface type.

Perennial taxa	# accns	# avail accns
<i>H. angustifolius</i>	19	6
<i>H. arizonensis</i>	5	2
<i>H. atrorubens</i>	14	8
<i>H. californicus</i>	21	15
<i>H. carnosus</i>	3	2
<i>H. ciliaris</i>	27	15
<i>H. cusickii</i>	21	12
<i>H. decapetalus</i>	31	23
<i>H. divaricatus</i>	32	9
<i>H. eggertii</i>	15	10
<i>H. floridanus</i>	4	2
<i>H. giganteus</i>	29	16
<i>H. glaucophyllus</i>	1	1
<i>H. gracilentus</i>	7	4
<i>H. grosseserratus</i>	44	40
<i>H. heterophyllus</i>	8	0
<i>H. hirsutus</i>	18	5
<i>H. laciniatus</i>	8	7
<i>H. xlaetiflorus</i>	7	2
<i>H. laevigatus</i>	6	2
<i>H. longifolius</i>	3	2
<i>H. maximiliani</i>	65	50
<i>H. microcephalus</i>	11	4
<i>H. mollis</i>	25	11
<i>H. xmultiflorus</i>	1	1
<i>H. nuttallii</i>	8	8
<i>H. nuttallii</i> ssp <i>nuttallii</i>	22	19
<i>H. nuttallii</i> ssp <i>rydbergii</i>	12	12
<i>H. occidentalis</i>	2	0
<i>H. occidentalis</i> ssp <i>occidentalis</i>	1	1
<i>H. occidentalis</i> ssp <i>plantagineus</i>	11	9
<i>H. pauciflorus</i>	11	6
<i>H. pauciflorus</i> ssp <i>pauciflorus</i>	6	5
<i>H. pauciflorus</i> ssp <i>subrhomboideus</i>	15	13
<i>H. pumilus</i>	51	44
<i>H. radula</i>	14	3
<i>H. resinosus</i>	16	11
<i>H. salicifolius</i>	1	0
<i>H. schweinitzii</i> *	1	1
<i>H. silphioides</i>	2	1
<i>H. simulans</i>	4	1
<i>H. smithii</i>	5	4
<i>H. strumosus</i>	34	17
<i>H. tuberosus</i>	107	35
<i>H. verticillatus</i>	2	2
total	750	441

*Species covered by the U.S. Fish and Wildlife Threatened and Endangered Species Act

Table 3. Summary of the USDA-NPGS *Helianthus* collection.

germplasm category	# of accessions	% of collection	% available, 2008
cultivated <i>Helianthus annuus</i> *	1702	44	93
wild <i>H. annuus</i>	931	24	96
wild annual non- <i>H. annuus</i>	455	12	85
wild perennial <i>Helianthus</i>	750	20	59
totals	3838	-	86

In order to develop molecular methods to assess within and between population variation, a collaborative effort is underway to associate molecular marker information with *Helianthus* accessions. Genotyping was initiated using *H. pumilus*, a perennial species sampled across its entire geographic range during a 2005 PEO sponsored collection trip to Colorado and Wyoming. This germplasm provides a broad sample of the species genetic diversity and makes it a good choice with which to begin molecular based diversity analyses. Data are being handled as molecular descriptors and following validation, will be available through GRIN.

DISCUSSION

Previous seed increase efforts at NCRPIS focused on ensuring high availability of cultivated *H. annuus* accessions. Discounting accessions not distributable due to Crop Science Registry restrictions, that portion of the collection is now greater than 95% available. Since 2004, efforts have focused on making wild germplasm more available for distribution both by increasing germplasm already in the collection and by organizing and participating in collection trips to obtain more complete genetic representation of the wild taxa in this native North American genus. Since 2004, availability of wild annual non-*Helianthus annuus* germplasm has increased 15% and wild perennial availability has more than tripled to 59%. Seed from wild populations can have varied dormancy requirements that affect germination. A protocol has been adopted that has enhanced our success with wild material, presuming adequate initial seed viability. Perennial species from the southern and southwestern United States and Mexico do not over-winter well in Ames; additionally, the growing season in Ames is too short to allow flowering and mature seed development for some of the wild taxa from these regions. To address some of these regeneration issues, we have developed a strong cooperative program with the NPGS site personnel in Parlier, CA with its longer growing season for the regeneration of up to 40 *Helianthus* accessions each year. Photoperiod control methods have been developed for use in Ames, IA.

In addition to routine field inspections to ensure quality seed increases, we also participate in select projects evaluating germplasm for disease resistance. One of the major diseases affecting sunflower in the United States is basal stalk rot, caused by *Sclerotinia sclerotiorum*. In 2007, screening of wild germplasm for resistance to *Sclerotinia* was begun as part of a joint effort with the USDA Sunflower Research Unit in Fargo, ND. Accessions of *H. resinosus* were notable in their resistance to infection both in greenhouse tests (greater than 99% for four accessions) and in the field. When greenhouse survivors were transplanted to the field and re-inoculated, survival remained high: 87-96% compared with approximately 20% for the resistant check hybrid. A long term goal is the incorporation of useful traits into cultivated germplasm.

The sunflower collection maintained by the NPGS is a very diverse collection. Sunflower germplasm is available for research and educational purposes at no charge. Samples may be obtained by ordering through GRIN or by contacting the senior author at lmarek@iastate.edu. A caveat for international requesters: NCRPIS must be able to meet any import order requirements stated by the requesting country. There are times when this is not possible and requests cannot be fulfilled.

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REFERENCES

- Marek, L. F., I. Larsen, C.C. Block, and C. Gardner. 2004. The sunflower collection at the North Central Regional Plant Introduction Station. p. 761-765. In: Proc. 16th Intl. Sunflower Conf., Fargo, ND, USA.
- Marek, L.F. 2008. Promoting flowering in *Helianthus argophyllus*: Manipulating daylength in the field. In: 28th Sunflower Research Workshop, January 10-11, 2008, Fargo, ND.
- Schilling, E.E. 2006. *Helianthus*. In: Flora of North America Editorial Committee (eds), 1993+. Flora of North America North of Mexico. 12+ vols. New York and Oxford. Vol. 21, p. 141- 200.