

2012 Update: New Sunflower Genetic Resources in the US National Sunflower Collection and Potential Use for Crop Improvement.

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ABSTRACT

- The *Helianthus* L. germplasm collection in the National Plant Germplasm System of the United States is held at the North Central Regional Plant Introduction Station in Ames, IA and administered by the USDA-ARS Plant Introduction Research Unit in cooperation with the Agricultural Experiment Station at Iowa State University. The sunflower collection is composed of accessions of the domesticated species *Helianthus annuus* and its wild relative taxa along with associated passport and other information. Sunflower germplasm is acquired, regenerated, characterized and distributed to conduct and support research and education. We provide a summary of regeneration protocols, describe new germplasm received in the past four years and provide an example of wild sunflower disease resistance evaluation taking place in Ames.
- Regenerations take place under controlled conditions so that the genetic character of received material is maintained as closely as possible. New material is obtained by donation, by receipt of material with expired intellectual property right protection and by active collection. The wild *Helianthus* collection is being systematically evaluated at Ames for resistance to *Sclerotinia* stalk rot using a sub-surface soil application of *Sclerotinia*-infested millet. Promising material is further evaluated in field trials to confirm resistance.
- Since 2008, three Plant Exchange Office (National Plant Genetic Resources Laboratory, USDA-ARS) sponsored collection trips for wild sunflower have added 160 accessions, sampling wild populations of 202 different taxa, two of which were not previously available for distribution. Additional accessions have been received by donation and after expiration of intellectual property right protection. Overall, 91% of the sunflower collection is available for distribution. Of accessions evaluated for *Sclerotinia* stalk rot in Ames, the best sources of resistance among annual species occur within *H. argophyllus*, *H. debilis*, *H. praecox* and *H. agrestis*, with little resistance found in wild *H. annuus*. The majority of accessions from the eight perennial taxa screened to date have shown superior resistance with 90-100% of the plants fully resistant to *Sclerotinia* stalk rot.
- The USDA sunflower collection provides a unique sampling of genetic diversity within the wild sunflower taxa as well as containing accessions of domesticated *H. annuus*. Wild sunflower accessions identified as resistant to *Sclerotinia* stalk rot during greenhouse-based screening in Ames have the potential to provide useful traits to improve cultivated sunflower.
- The USDA sunflower collection is freely available to scientists and educators world-wide for research, crop improvement, product development and educational use.

Key words: cultivated - germplasm collection - *Helianthus* – wild – *Sclerotinia*

INTRODUCTION

The *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 which is administered by the USDA-ARS Plant Introduction Research Unit in cooperation with the Agricultural Experiment Station at Iowa State University. The NCRPIS sunflower collection contains accessions for all 52 *Helianthus* species recognized in the recent Flora of North America (FNA) treatment of *Helianthus* (Schilling, 2006), including accessions of the domesticated annual species *Helianthus annuus*. Thirty-eight of the species are perennial and 14 are considered annual. The genus *Helianthus* originated in North America and is well distributed across the United States, although a number of *Helianthus* taxa have restricted ranges. Two species, *H. paradoxus* and *H. schweinitzii*, are on the U.S. Fish and Wildlife Service's Threatened and Endangered Species List. The NCRPIS *Helianthus* collection provides a valuable genetic resource for the improvement of cultivated sunflower. Basal stalk rot, caused by *Sclerotinia sclerotiorum*, is one of the major diseases affecting sunflower in the United States. Resistance to *Sclerotinia* stalk rot had been observed in cultivated accessions in the NCRPIS collection. In working toward obtaining a more comprehensive resistance, we have been evaluating wild sunflower accessions for their response to this problematic pathogen. In this paper, we provide a summary of sunflower regeneration protocols in use at the NCRPIS, describe new germplasm received in the past four years and provide an example of a wild sunflower disease evaluation taking place in Ames.

MATERIALS AND METHODS

Regenerations: All sunflower accessions are regenerated under conditions of controlled pollination to maintain the genetic integrity of the original material. Seeds of wild accessions are soaked in 3% H₂O₂ for 5 min, then rinsed with cool, running tap water and placed in small beakers containing 25mg/kg ethephon (Ethrel) solution. Seeds are soaked at room temperature overnight. The next day, the seeds are rinsed again with cool, running tap water and transferred to germination boxes. Seeds are incubated for at least 2 and up to 8 weeks at 4C 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 transferred to flats and established in the greenhouse before being transplanted to the field. Wild accessions are caged and screened before flowering and honey bees are introduced into the cages for pollination after flowering begins. A number of wild taxa require a longer growing season than reliably occurs in central Iowa. We maintain a significant partnership with the NPGS Parlier, CA location in the San Joaquin Valley (USDA-ARS National Arid Land Plant Genetic Resource Unit, NALPGRU) to regenerate these taxa. We germinate seeds in Ames and ship seedlings 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. All regenerations of cultivated *H. annuus* accessions take place in Ames and are managed by direct seeding into rows except for those accessions with low quantity or quality seed which are started in incubators, transferred to the greenhouse, and transplanted to the field. Cultivated accessions are either hand pollinated after bagging the primary inflorescence or, in the case of accessions with branching, rows are arranged so the plants can be caged, screened and pollinated with honey bees with the wild accessions. Since 1990, regeneration fields have been inspected during the growing season by the NCRPIS pathology team, as described in Marek, et al., 2008, allowing many disease-free declarations to be met for international shipment. After harvesting, drying and cleaning, seeds are stored at 4C and 35% humidity.

New Germplasm: Since September 2008, the NPGS Plant Exchange Office (PEO) has sponsored three collection trips organized by Dr. Marek to the southeastern (2008; Georgia, South Carolina, North Carolina, Tennessee), south central (2009; Missouri, Kansas, Oklahoma, Arkansas), and southern (2010; Louisiana, Mississippi, Alabama, Florida) United States during which 160 populations of 20 different wild sunflower taxa were sampled (Table 1). Dr. Gerald Seiler (USDA Research Botanist, USDA Sunflower Research Unit, Fargo, ND), participated in these collection efforts and added taxonomic expertise. Thirty-nine additional wild collections of 17 taxa were donated by 10 independent collaborators, including eight *H. argophyllus* accessions from naturalized wild populations in Australia. Since mid-2008, 122 cultivated accessions have been released to the active NCRPIS collection, primarily expired Crop Science Registry (CSR) materials.

Disease evaluation: Wild sunflower taxa are evaluated for their response to *Sclerotinia sclerotiorum* as follows. Wild sunflower seeds are germinated as described for regenerations above except that a 1% sodium hypochlorite rinse is used after the ethephon soak to help control seed pathogens and no H₂O₂ is used. Germinated seeds are transplanted to 32-cell greenhouse flats (11x21 cm) and seedlings are grown under a 14-hr daylength until they reach the 6-8 leaf stage. Seedlings are inoculated by transferring them

to 32-cell flats containing a 2.5g basal layer of Sclerotinia-infested millet. Temperatures in the greenhouse are managed to maintain soil temperatures between 21 to 23C. Populations of 60 plants have proven to give an adequate assessment of resistance within an accession. The 60 plants of each accession are arranged in a randomized complete block layout with 15 replications of 4 plants per replication. Plants are observed daily and days to first wilt and to permanent wilt are recorded. The experiment is stopped after 21 days and remaining live plants noted. Seedlings of one highly susceptible commercial hybrid and two or three moderately resistant hybrids are used as controls in each experiment. Results are expressed as accessions showing resistance superior to the most resistant hybrid control.

RESULTS

Regeneration protocols for wild and cultivated sunflower accessions have evolved over the years. The protocol described in this paper has provided consistent results over the past two years and has helped us reliably manage regenerations in Ames and produce seedlings for regenerations at the NALPGRU, Parlier, CA. The NCRPIS collection currently contains 4,032 accessions, 3,200 annual (Table 1) and 832 perennial (Table 2) accessions, representing all of the currently recognized *Helianthus* species (Schilling, 2006). We have continued to fill gaps in the collection striving to obtain samples from populations across the geographic distributions of each taxon through targeted collection efforts. Two perennial species not previously represented by viable accessions were re-collected during the 2008 – 2010 PEO sponsored collection trips and are now available for distribution (Table 2).

Table 1. Annual *Helianthus* taxa in the NCRPIS collection.

Annual taxa	# accessions at NCRPIS	# available accessions	new accessions since fall 2008
<i>H. agrestis</i>	10	9	7
<i>H. annuus</i> , cultivated	1816	1725	122
<i>H. annuus</i> , wild	933	913	7
<i>H. anomalus</i>	6	6	1
<i>H. argophyllus</i>	49	46	10
<i>H. bolanderi</i>	7	5	
<i>H. debilis</i>	1	0	1
<i>H. debilis</i> ssp <i>cucumerifolius</i>	11	11	
<i>H. debilis</i> ssp <i>debilis</i>	12	10	
<i>H. debilis</i> ssp <i>silvestris</i>	22	21	
<i>H. debilis</i> ssp <i>tardiflorus</i>	5	4	
<i>H. debilis</i> ssp <i>vestitus</i>	3	3	
<i>H. deserticola</i>	21	15	
<i>H. exilis</i>	30	30	
<i>H. neglectus</i>	28	28	
<i>H. niveus</i>	1	1	
<i>H. niveus</i> ssp <i>canescens</i>	19	15	
<i>H. niveus</i> ssp <i>tephrodes</i>	10	8	
<i>H. paradoxus</i> *	2	2	
<i>H. petiolaris</i>	15	15	
<i>H. petiolaris</i> ssp <i>fallax</i>	30	30	
<i>H. petiolaris</i> ssp <i>petiolaris</i>	93	93	
<i>H. porteri</i>	9	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>	9	8	
<i>H. hybrid</i>	17	15	
total	3200	3062	148

*species listed on the U.S. Fish and Wildlife Service Threatened and Endangered Species Act

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

Perennial taxa	# accessions at NCRPIS	# available accessions	new accessions since fall 2008
<i>H. angustifolius</i>	26	17	12
<i>H. arizonensis</i>	2	2	
<i>H. atrorubens</i>	14	13	2
<i>H. californicus</i>	21	16	
<i>H. carnosus</i>	2	2	
<i>H. ciliaris</i>	27	14	1
<i>H. cusickii</i>	20	19	
<i>H. decapetalus</i>	30	24	
<i>H. divaricatus</i>	27	15	
<i>H. eggertii</i>	13	11	
<i>H. floridanus</i>	9	6	5
<i>H. giganteus</i>	25	19	1
<i>H. glaucophyllus</i>	12	8	10
<i>H. gracilentus</i>	6	6	
<i>H. grosseserratus</i>	44	40	
<i>H. heterophyllus</i>	17	11	17
<i>H. hirsutus</i>	12	6	
<i>H. laciniatus</i>	7	7	
<i>H. xlaetiflorus</i>	11	8	4
<i>H. laevigatus</i>	7	4	3
<i>H. longifolius</i>	3	3	
<i>H. maximiliani</i>	64	51	2
<i>H. microcephalus</i>	13	8	4
<i>H. mollis</i>	27	12	4
<i>H. nuttallii</i>	8	8	
<i>H. nuttallii</i> ssp <i>nuttallii</i>	22	20	1
<i>H. nuttallii</i> ssp <i>rydbergii</i>	12	12	
<i>H. occidentalis</i>	2	1	
<i>H. occidentalis</i> ssp <i>occidentalis</i>	1	1	
<i>H. occidentalis</i> ssp <i>plantagineus</i>	12	9	3
<i>H. pauciflorus</i>	11	10	
<i>H. pauciflorus</i> ssp <i>pauciflorus</i>	21	14	16
<i>H. pauciflorus</i> ssp <i>subrhomboideus</i>	14	14	
<i>H. pumilus</i>	52	46	1
<i>H. radula</i>	40	26	33
<i>H. resinosus</i>	24	18	12
<i>H. salicifolius</i>	19	18	19
<i>H. schweinitzii</i> *	1	1	
<i>H. silphioides</i>	15	12	15
<i>H. simulans</i>	5	5	4
<i>H. smithii</i>	7	6	2
<i>H. strumosus</i>	33	21	
<i>H. tuberosus</i>	92	41	
<i>H. verticillatus</i>	2	2	
total	832	607	171

*species listed on the U.S. Fish and Wildlife Service Threatened and Endangered Species Act

We have been systematically evaluating wild sunflower taxa for their response to *Sclerotinia sclerotiorum* in the greenhouse to identify promising material for further investigations. The greenhouse efforts allow evaluation of ten times as many accessions than a single season of field based screening would allow as well as provide a more consistent and standard initial disease pressure. We have observed resistance superior to our most resistant hybrid control in accessions in more than half (7 of 13) of the annual species evaluated (Table 3). Overall, we observed much less resistance among the annual species (16% of 439 accessions tested were superior to the most resistant hybrid control) than in the perennial species (all accessions of all species evaluated have shown superior resistance), except that all accessions of the annual species *H. agrestis* tested to date have shown superior resistance (Table 3).

Table 3. Results of greenhouse evaluations for *Sclerotinia* basal stalk rot resistance in accessions of 13 annual (A) and 8 perennial (B) wild *Helianthus* species.

A. Annual species		
Taxon	# accessions screened	Accessions superior to most resistant hybrid control
<i>H. annuus</i> , wild	99	0 (0%)
<i>H. agrestis</i>	3	3 (100%)
<i>H. anomalus</i>	4	0 (0%)
<i>H. argophyllus</i>	30	16 (53%)
<i>H. bolanderi</i>	5	0 (0%)
<i>H. debilis</i>	45	7 (16%)
<i>H. exilis</i>	26	1 (4%)
<i>H. deserticola</i>	10	0 (0%)
<i>H. neglectus</i>	28	12 (43%)
<i>H. niveus</i>	9	0 (0%)
<i>H. paradoxus</i>	2	0 (0%)
<i>H. petiolaris</i>	137	24 (18%)
<i>H. praecox</i>	41	9 (22%)
annual totals (13)	439	72 (16%)
B. Perennial species		
Taxon	# accessions screened	Accessions superior to most resistant hybrid control
<i>H. californicus</i>	10	10 (100%)
<i>H. ciliaris</i>	4	4 (100%)
<i>H. eggertii</i>	5	5 (100%)
<i>H. grosseserratus</i>	37	37 (100%)
<i>H. pauciflorus</i>	31	31 (100%)
<i>H. resinosus</i>	14	14 (100%)
<i>H. salicifolius</i>	14	14 (100%)
<i>H. tuberosus</i>	38	38 (100%)
perennial totals (8)	153	153 (100%)

DISCUSSION

Regeneration efforts at the NCRPIS have ensured a high availability of the sunflower collection. Cultivated *H. annuus* accessions are 95% available for distribution, wild *H. annuus* accessions are 98% available and accessions of all other wild annual species are 94% available for distribution. We have been managing a steady increase in the availability of wild perennial species and perennial accessions are

now 73% available, representing an increase in both number of species and number of accessions available for distribution since 2008 when perennial availability was 59%. The strong cooperative program we have developed with the NALPGRU in Parlier, CA has been a critical component in our successful work with many of the wild species. Targeted collection efforts have increased the number of wild species with available accessions. A primary target of the 2008 collection trip was *H. heterophyllus* in its northern range and in 2010 we targeted it in its southern distribution range making this wild species available for distribution from the NCRPIS for the first time. A primary target of our 2009 collection trip, *H. salicifolius*, had never before been available for distribution, despite the fact that populations of this species are relatively common in the geographic area covered by eastern Kansas, western Missouri and northeastern Oklahoma where it is native. The NCRIPS sunflower collection provides a rich resource of genetic diversity to be evaluated for useful traits to incorporate into cultivated sunflower. The disease evaluation underway at Ames is an example of the first steps in this incorporation process.

One of the major diseases affecting sunflower in the United States is basal stalk rot, caused by *Sclerotinia sclerotiorum*. During greenhouse evaluations of 21 different wild species of *Helianthus* for resistance to stalk rot, we observed a consistent response of virtual immunity by all accessions of perennial species. The wild annual species showed much less consistency in their response to the strong disease pressure provided by the method described here. Six of the thirteen annual species evaluated exhibited zero resistance in all tested accessions. Six of the remaining tested species had 4-53% of tested accessions exhibit resistance superior to the most resistant control. Only one annual species, *H. agrestis*, had all tested accessions exhibit superior resistance. This variation in response suggests the possibility that different mechanisms of resistance could be involved. It is especially interesting that *H. agrestis* is the only annual species showing a consistent superior resistance. *H. agrestis* has other unusual characteristics including high self-fertility (all other wild *Helianthus* species are self-incompatible; Heiser, 1969) and chromosomes several times larger than those in all other *Helianthus* species. We have collected from additional populations of *H. agrestis* and samples from these collections will be included in our ongoing evaluation efforts to determine if these populations also exhibit superior resistance. The diversity present in the wild sunflower germplasm collection may provide a means to dissect the disease response of *Helianthus* to *Sclerotinia sclerotiorum* and allow us to reach a long term goal of the incorporation of useful traits (ie, disease resistance) from the wild species 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 the NPGS database GRIN (<http://www.ars-grin.gov/npgs/index.html>) or by contacting the senior author at Imarek@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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