EXPLORATION FOR WILD *HELIANTHUS* SPECIES IN NORTH AMERICA: CHALLENGES AND OPPORTUNITIES IN THE SEARCH FOR GLOBAL TREASURES

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Abstract

The genus *Helianthus* has 51 species, 14 annual and 37 perennial. The wild sunflower species have contributed many agronomically important traits to cultivated sunflower. The estimated economic value of their contribution to cultivated sunflower varies from $269.5 to $384 million annually. The USDA-ARS formally established a wild *Helianthus* germplasm collection at Bushland, Texas, in 1976. Having the wild species of *Helianthus* within the boundaries of the USA has facilitated the collection of sunflower germplasm. The explorations for wild sunflowers over the past 30 years have resulted in the assemblage of a USDA-ARS collection that is the most complete collection in the world. It is presently located at the National Plant Germplasm System, Plant Introduction Station at Ames, Iowa. Currently, the wild *Helianthus* collection contains 2163 accessions, about two-thirds of which are annual species. Aggressive collection of wild sunflower germplasm for preservation in seed banks is critical so that germplasm may be readily available to the sunflower genetics and the breeding community. Furthermore, given the tenuous situation of wild species populations in nature, seed banks may provide the only way to preserve some wild populations or species for posterity. The genus *Helianthus* is an extremely diverse group of species whose geographic distribution ranges from nearly universal in all the continental United States, to species which are found only in few locations or in threatened habitats. While we have representative populations of most species, we do not have the total genetic diversity available. There is a need to collect additional populations of wild sunflower species. Currently, 37 of the 65 taxa are either not available due to low seed supplies or have few accessions for research. Future plans are to systematically add species populations to the germplasm collection. Assuming one collecting trip per year, it may be possible to collect seed of all remaining 36 taxa within the next decade. The sunflower research community has an opportunity to collect and preserve the unique genetic resources of the wild relatives of *Helianthus* and to pass it on to future generations to be used for improving cultivated sunflower.

Introduction

Plant genetic resource (or simply germplasm) management comprises two phases. The first, germplasm conservation, includes acquisition, or securing germplasm in situ (by establishing reserves) or ex situ (by assembling collections through exchange or exploration and placing seeds in gene banks (Bretting and Widrlechner, 1995). Genetic resources of a crop consist of the total pool of genetic variability that exists in the crop species or within species with which the crop plant is sexually compatible (Holden et al., 1993). Sunflower germplasm resources can be categorized as in situ resources (i.e., wild populations) or ex situ resources (accessions preserved in seed banks, which can include the wild species).

Genes that encode heritable characters of populations and plants often vary among plants within species, with greater genic differences typically occurring between species. The continual reordering of genes through sexual reproduction and mutations may modify existing genes and/or their expression or generate new genes, creating the variation that enables plants to grow and survive in diverse environments or adapt to stresses. The magnitude of genic variation within a given population of plants is described by the term genetic diversity.

Wild relatives of crop plants typically are genetically much more diverse than related cultivated lineages. Genetic diversity is thought to contribute to long-term preservation of species by allowing them to quickly adapt to biotic and abiotic changes in their environment. Diversity in germplasm is also critical to crop breeding programs, but this has not been fully exploited (Harlan, 1976). Although many germplasm introductions appear to have no immediate use in breeding and genetic programs (Burton, 1979), they may contain unidentified genes that will protect crops against future pests. Thus, if new pests or environmental stresses extend beyond normal limits of tolerance, productivity loss occurs, and a search for germplasm with greater resistance to the stresses is initiated. Hopefully, the current germplasm collection will contain the necessary germplasm. Although we cannot predict with acceptable levels of confidence the occurrence, severity, or even the nature of future stresses, germplasms with as much genetic diversity as possible should be available for breeding programs (Jones, 1983).

Exploration and collection of germplasm represents one of the more difficult and challenging activities in the process of conserving genetic diversity in the genus *Helianthus.* Chang (1985) listed several obstacles to overcome in germplasm explorations, including: 1) overcoming physical hardships and obstructions to locate populations that commonly are located in remote habitats, 2) finding wild species habitats, proper species identification, and employing appropriate sampling strategies to obtain maximum genetic diversity, 3) explorations are time-consuming and expensive, 4) explorations are usually too short to carry out all the desired prospective collecting, 5) locating an earlier documented collecting site for follow-up collection is not always possible, and 6) previous collection sites may have been destroyed before subsequent explorations.

The exploration and collection of wild sunflower species to preserve them for future use presents several interesting challenges and opportunities. We will discuss the exploration process from the planning stages through the collection stages and describe the collection needs and the potential value and uniqueness of the acquired germplasm.

Discussion

Generic Description. The identification of sunflower species has long been problematic. Heiser et al. (1969) felt that the greatest contribution of his sustained efforts to understand sunflower taxonomy was not providing an easy way to identify sunflowers, but rather an explanation for why they are so difficult. The taxonomic complexity of the genus *Helianthus* stems from many different factors. Natural hybridization and introgression occur between many of the species, often resulting in morphological intergradation between otherwise distinct forms. Polyploidy in the perennial species also contributes to the complexity of species classification in *Helianthus*. This has led to various taxonomic treatments of the genus. There are still specimens, variously of hybrid origin or growing in unusual conditions or incompletely collected, that defy certain placement into a single species (E.E. Schilling, pers. com., 2003). Since many of the species are wide-ranging geographically, they exhibit extensive phenotypic variation, which appears to include both heritable and non-heritable (environmental) components. Many species are also genetically quite variable, making rigorous identification and classification difficult.

The genus *Helianthus* has been considered to comprise from as few as 10 species to more than 200. Linnaeus (1753) originally described nine species in the genus. Asa Gray (1889) recognized 42 species in North America. In the early 20th century, Watson (1929) accepted 108 species, 15 of them from South America. Heiser et al. (1969) recognized 14 annual species and 36 perennial species from North America in three sections and seven series, as well as 17 species from South America. Subsequently, Robinson (1979) transferred 20 perennial species of South American *Helianthus* to the genus *Helianthopsis.* The taxonomic classification of *Helianthus* by Anashchenko (1974, 1979) was a radical departure from all previous schemes. He recognized only one annual species, *H. annuus* (with three subspecies and six varieties), and only nine perennial species with 13 subspecies. Schilling and Heiser (1981) proposed an infrageneric classification of *Helianthus,* using phenetic, cladistic, and biosystematic procedures that places 49 species of *Helianthus* in four sections and six series (Tables 1 and 2). The classification of Schilling and Heiser (1981) is presented herein with the following six modifications. First, the sectional name *Atrorubens* used by Anashchenko (1974) has taxonomic priority, thus the section *Divaricati* E. Schilling and Heiser is replaced by section *Atrorubens* Anashchenko. Second, *Helianthus exilis* is recognized as a species, as opposed to an ecotype of *H. bolanderi* due to recent information which has shown it to be morphologically and genetically distinct (Oliveri and Jain, 1977; Rieseberg et al., 1988; Jain et al., 1992). Third, the species name *H. pauciflorus* has priority over *H. rigidus* and is treated accordingly herein. Fourth, *Viguiera porteri* has been transferred to *Helianthus porteri* (Pruski, 1998; Schilling et al., 1998). Fifth, *Helianthus verticillatus* has recently been rediscovered and redescribed and is now recognized as a species (Matthews et al., 2002). Sixth, *Helianthus niveus* ssp. *canescens* has been transferred to *Helianthus petiolaris* ssp. *canescens* (Schilling, pers. com., 2003). This brings the number of species to 51, with 14 annual and 37 perennial (Tables 1 and 2).

Table 1. Infrageneric classification of annual *Helianthus* species.

Section*	Species	Common Name	Chromosome Number (n)
Helianthus	*H. annuus* L.	Prairie	17
	H. anomalus Blake	Anomalous	17
	H. argophyllus T.& G.	Silver-leaf	17
	H. bolanderi A. Gray	Bolander's, Serpentine	17
	H. debilis		
	ssp. *debilis* Nutt.	Beach	17
	ssp. *cucumerifolius* (T. & G.) Heiser	Cucumber leaf	17
	ssp. *silvestris* Heiser	Forest	17
	ssp. *tardiflorus* Heiser	Slow-Flowering	17
	ssp. *vestitus* (Watson) Heiser	Clothed	17
	H. deserticola Heiser	Desert	17
	H. exilis A. Gray	Serpentine	17
	H. neglectus Heiser	Neglected	17
	H. niveus		
	ssp. *niveus* (Benth.) Brandegee	Snowy	17
	ssp. *tephrodes* (Gray) Heiser	Ash-Colored, Dune	17
	H. paradoxus Heiser	Pecos, Puzzle, Paradox	17
	H. petiolaris		
	ssp. *canescens* (A. Gray) E. E.Schilling	Gray	17
	ssp. *fallax* Heiser	Deceptive	17
	ssp. *petiolaris*	Prairie	17
	H. praecox		
	ssp. *hirtus* Heiser	Texas	17
	ssp. *praecox* Englm. & A.Gray	Texas	17
	ssp. *runyonii* Heiser	Runyon's	17
Agrestes	*H. agrestis* Pollard	Rural, Southeastern	17
Porteri	*H. porteri* (A. Gray) J. F. Pruski	Confederate Daisy, Porter's	17

(*Schilling and Heiser, 1981; E.E. Schilling, pers. com., 2003).

Table 2. Infrageneric classification of perennial *Helianthus.*

Section*	Series	Species	Common Name	Chromosome Number (n)
Ciliares	*Ciliares*	*H. arizonensis* R. Jackson	Arizona	17
		H. ciliaris DC.	Texas blueweed	34, 51
		H. laciniatus A. Gray	Alkali	17
Ciliares	*Pumili*	*H. cusickii* A. Gray	Cusick's	17
		H. gracilentus A. Gray	Slender	17
		H. pumilus Nutt.	Dwarfish	17
Atrorubens	*Corona-solis*	*H. californicus* DC.	California	51
		H. decapetalus L.	Ten-petal	17, 34
		H. divaricatus L.	Divergent	17
		H. eggertii Small	Eggert's	51
		H. giganteus L.	Giant	17
		H. grosseserratus Martens	Sawtooth	17
		H. hirsutus Raf.	Hairy	34
		H. maximiliani Schrader	Maximilian's	17
		H. mollis Lam.	Soft, Ashy	17
		H. nuttallii ssp. *nuttallii* T. and G.	Nuttall's	17
		H. nuttallii ssp. *rydbergii* (Brit.) Long	Rydberg's	17
		H. resinosus Small	Resinous	51
		H. salicifolius Dietr.	Willow-leaf	17
		H. schweinitzii T. and G.	Schweinitz's	51
		H. strumosus L.	Swollen, Woodland	43, 51
		H. tuberosus L.	Jerusalem artichoke	51
Atrorubens	*Microcephali*	*H. glaucophyllus* Smith	White-leaf	17
		H. laevigatus T. and G.	Smooth	34
		H. microcephalus T. and G.	Small-headed	17
		H. smithii Heiser	Smith	17, 34
Atrorubens	*Atrorubentes*	*H. atrorubens* L.	Purple-disk	17
		H. occidentalis ssp. *occidentalis* Riddell	Few-leaf, Western	17
		H. occidentalis ssp. *plantagineus* (T. & G.) Heiser	Few-leaf, Western	17
		H. pauciflorus ssp. *pauciflorus*	Stiff	51
		H. pauciflorus ssp. *subrhomboides* (Rydb.) O. Spring	Stiff	51
		H. silphioides Nutt.	Odorous	17
Atrorubens	*Angustifolii*	*H. angustifolius* L.	Narrow-leaf, Swamp	17
		H. carnosus Small	Fleshy	17
		H. floridanus A. Gray ex Chapman	Florida	17
		H. heterophyllus Nutt.	Variable-leaf	17
		H. longifolius Pursh	Long-leaf	17
		H. radula (Pursh) T. and G.	Scraper, Rayless	17
		H. simulans E. E. Wats.	Muck, Imitative	17
		H. verticillatus Small	Whorled	17

(*Schilling and Heiser, 1981; Schilling, pers. com., 2003).

Economic Value of the Wild Species. Wild species have contributed many agronomically important traits to cultivated sunflower. The estimated economic value of the contribution of the wild species to cultivated sunflower is $384 million per year (Prescott-Allen and Prescott-Allen, 1986). Another estimate is $269.5 million per year (Phillips and Meilleur, 1998). The greatest value is derived from the PET1 cytoplasmic male sterile (CMS) cytoplasm from *H. petiolaris*. Wild *Helianthus* species have been an important and significant source of genes for cultivar resistance to economically important pathogens. Over the past several decades genes for resistance to several diseases, such as rust (Quresh et al.,1993; Gulya et al., 2000), downy mildew (Tan et al., 1992; Jan et al., 1991; Seiler, 1991, 1998; Seiler and Gulya, 1992; Miller et al., 2002), powdery mildew (Jan and Chandler, 1985, Rojas-Barros et al., 2003), broomrape (Jan and Fernández-Martínez, 2002, Jan et al., 2002a) and Sclerotinia head rot (Rashid and Seiler, 2001, 2003; Mondolot-Cosson and Andary, 1994), Sclerotinia stalk rot (Seiler et al., 1993) and resistance to insects such as sunflower moth (Rogers et al., 1984) and sunflower beetle (Roseland and Seiler, 1993) have been identified in wild *Helianthus* species and successfully transferred to cultivated sunflower. Other useful traits found in wild *Helianthus* species include cytoplasmic male sterility (Jan, 2000; Jan et al., 2002b), altered fatty acid composition (Seiler, 1996c, 2002), and salt tolerance (Miller, 1995; Miller and Seiler, 2004). One trait not accounted for in the dollar estimates mentioned above is herbicide resistance. A wild population of *H. annuus* L. from Kansas has been identified as a source of genes for resistance to imidazolinone and sulfonylurea herbicides and its resistance genes have been transferred into cultivated sunflower (Al-Khatib et al., 1998; Al-Khatib and Miller, 2000). In addition, these two herbicides control broomrape in areas of the world where this parasitic weed is prevalent (Alonso et al., 1998). Thus, herbicide resistant sunflower hybrids could be combined with herbicides to combat broomrape infection.

More detailed discussions about the use and potential value of wild species for sunflower breeding programs can be found in Seiler (1984, 1992, 1996, 2002), Seiler and Rieseberg (1997), and Skoric (1988, 1992).

Wild Species Concerns. Preservation of wild sunflower species populations is critical because we lack the resources necessary to preserve all wild species and locally adapted sunflower populations in seed banks. Furthermore, a significant proportion of wild diversity would likely be lost while regenerating banked germplasm accessions. Unfortunately, the long-term outlook for survival of a number of sunflower species is not promising; some species already are rare and endangered, or in the case of *H. nuttallii* T. and G. ssp. *parishii* (A. Gray) Heiser, probably extinct. Our efforts to conserve the wild sunflower species will have to be a combination of preserving some of the species as populations in nature and also collecting of seeds for gene bank preservation.

The U.S. Department of Interior, Fish and Wildlife Service has listed four species of *Helianthus* under the Endangered Species Act of 1973 as threatened, endangered or a candidate species for listing (USDI, Fish and Wildlife Service, 2003). Annual *H. paradoxus* (Pecos, puzzle, paradox sunflower) is a threatened species which is restricted in distribution to New Mexico and Texas. In 1980 there were only a few populations of this endemic species known from its specialized moist saline habitat requiring permanent wetlands for survival. The discovery of a large population of this species in Texas and one in New Mexico in 1980 assured the potential survival of this species (Seiler et al., 1981). Since that time, the Pecos sunflower can now be found at 25 sites in five areas of New Mexico and Texas (Nevarez, 2002).

The perennial species *H. eggertii* Small (Eggert's sunflower) with a current range of Alabama (one county), central Kentucky (six counties), and central Tennessee (13 counties) is also listed as threatened. It is known from an estimated 200 populations. The primary habitats are open barrens or open oak-hickory woods on rolling to flat topography, on well-drained, shallow, acidic soils, underlain by limestone (Jones, 1994). It is threatened throughout its range by habitat alterations, residential, commercial, or industrial development, plant succession, and conversion of its limited habitat to pastures and croplands. Herbicide use, particularly along roadsides also poses a threat. Recovery strategies developed for Eggert's sunflower by the US Fish and Wildlife Service call for the enhancement and maintenance of populations through habitat protection, management, and restoration (White and Ratzlaff, 1999).

Schweinitz's sunflower (*H. schweinitizii)* is listed as endangered and restricted to the Piedmont of North and South Carolina and is included in an in situ recovery plan to enhance its survival (Weakley and Houk, 1993). Fifty-four populations are known, 38 from North Carolina and 16 from South Carolina. Its typical habitat includes roadsides, power line clearings, old pastures, woodland openings, and other sunny to semi-sunny places. It is generally located on poor, clayey and/or rocky soils, especially those derived from mafic rocks. Formerly it probably occurred in prairie habitats or post oak-blackjack oak savannas maintained by fires set by lightning and Native Americans.

Whorled sunflower (*Helianthus verticillatus* Small) has been recently rediscovered and redescribed (Allison, 1997; Mathews et al., 2002). In 1898, the species was first discovered in Chester County, Tennessee by Samuel McCutcheon, a botanist at the University of Tennessee. In 1994, almost one hundred years later, another population was found in Floyd County, Georgia. Recently, two more populations were found, one in Cherokee County, Alabama, and another one in Madison County, Tennessee. It is currently listed as a candidate species for further review by the US Fish and Wildlife Service. The general habitat is moist prairie-like openings, woodlands, and sandy clays that are alkaline and wet. *Helianthus verticillatus* appears to have restricted ecological requirements and is dependent upon the maintenance of prairie-like openings for its survival. Much of this species habitat has been degraded due to fire suppression and the subsequent invasion of woody competitors. Extant sites will require active management to keep competition and shading under control.

Serpentine sunflower, *Helianthus exilis*, had been listed by California as a category 3 (threatened) species, and this classification spurred state and federal agencies to monitor its occurrence. It is restricted to dry serpentine barrens, dry serpentine creek beds, and serpentine seeps at elevations of 100 to 1400 meters in California. Serpentine soils are nutritionally very poor, but certain plants have adapted to these barren soils. Currently 60 sites are known for this species. Many of the populations are in remote areas and are on state or federal forest lands so monitoring of populations will be possible. Because of its putative introgressive relationship with *H. bolanderi*, some authors have considered *H. bolanderi* and *H. exilis* to be closely related introgressive races or ecotypes. Thus, *H. exilis* has been considered a synonym of *H. bolanderi* in recent taxonomic treatments of the annual sunflowers (Heiser, 1949, 1978; Heiser et al., 1969; Schilling and Heiser, 1981). However, the two taxa show considerable divergence in morphology, physiology, fatty acid composition (Oliveri and Jain, 1977; Jain et al., 1977; Rogers et al., 1982), and chloroplast DNA sequence (Rieseberg et al., 1988). Therefore, *H. exilis* is recognized as a distinct species, in concurrence with Oliveri and

Jain (1977); Jain et al. (1992); Rogers et al. (1982); Rieseberg (1991b); and Schilling, pers. com., 2003).

The primary obstacle for long-term preservation of wild sunflower populations is human activity. For example, the marshy habitat of *H. nuttallii* ssp. *parishii* in southern California has been completely eliminated and replaced by urban development. Also, the widening of highways and their rights-of-way in Texas has apparently eliminated populations of *H. paradoxus* and *H. praecox* ssp. *hirtus*, and mining activities in California have destroyed several populations of *H. exilis*. In addition to the extinction of populations by development, their disturbance by humans can lead to hybridization between widespread species and the resulting recent introduction of more widespread congeners (Rieseberg, 1991b). Not only are the hybrid plants likely to be less fit than locally adapted populations, but populations of rare species may be genetically "swamped" out of existence by populations of the numerically larger introduced species. It is noteworthy that the common sunflower, *H. annuus*, occurs sympatrically and hybridizes with several rare annual sunflowers (e.g., *H. paradoxus, H. anomalus*, and *H. deserticola)*, possibly posing a threat to their existence or genetic integrity.

Other potential threats to the preservation of rare sunflower species populations include their small population sizes and subsequent loss of genetic diversity. Isozyme analyses of populations of annual sunflowers revealed a strong correlation between their genetic diversity and geographic range (Rieseberg et al., 1991a). In fact, 8 of the 11 narrow endemics had lower levels of genetic diversity than any of their more widespread congeners. In particular, very low levels of genetic diversity were observed for *H. paradoxus, H. deserticola, H. debilis* ssp. *tardiflorus*, and *H. debilis* ssp. *vestitus*. Although these values may be cause for concern, it should be pointed out that links among genetic diversity, fitness, and evolutionary potential are not apparent.

The hybrid origin of *H. anomalus, H. deserticola* and *H. paradoxus* from their parental species *H. annuus* and *H. petiolaris* presents an interesting challenge (Rieseberg, 1991a; Rieseberg et al., 2003). The specific habitats for these species make them especially vulnerable, pointing out the need to collect and closely monitor these species (Schwarzbach et al., 2001; Rosenthal et al., 2002; Gross et al., 2003).

Exploration. Aggressive collection of wild and domesticated sunflower germplasm for preservation in seed banks is critical so that germplasm may be readily available to the sunflower genetics and breeding community. Furthermore, given the tenuous situation of wild populations in nature, seed banks may provide the only way to preserve some wild populations or species for posterity.

Having the wild species of *Helianthus* within the boundaries of the USA has facilitated the collection of sunflower germplasm. The value of the wild progenitors as potential sources of genes for disease and insect resistance for cultivar improvement was recognized early (Pustovoit et al., 1976). This soon led to efforts to collect and use wild *Helianthus* germplasm. Dr. Charles Heiser of Indiana University was one of the early collectors of *Helianthus* germplasm. He began collecting in 1947 (C. Heiser, pers. com., 2001). His focus was primarily taxonomy, systematics, and evolution and speciation of the genus. His early work formed the basis of the current knowledge and understanding of the *Helianthus* genus.

Early explorations for rust-resistant germplasm were undertaken by Drs. Murray Kinman (USDA-ARS, College Station, TX) and Aurelia Luciano (Argentina) in Texas and Oklahoma in 1963 (Seiler, 1988b). The wild species most represented was wild *H. annuus* which was introgressed into cultivated sunflower and formed the basis for restoration lines for many of the first hybrids in the USA and around the world.

During the 1970s, Dr. Ben Beard (USDA-ARS, Davis, CA) collected wild sunflowers throughout the southwestern USA. There were approximately 200 accessions in this collection (Seiler, 1988b). Most of the accessions were annual species, with many wild *H. annuus* accessions.

Another exploration for sources of rust resistance and a survey of rust races in the North Central Great Plains was undertaken in 1972 by Gerald Seiler (Zimmer and Rehder, 1976). This exploration added 100 accessions of mostly wild annual species, predominantly *H. annuus*, to the wild species collection.

Prior to 1976, the wild species collection consisted of some 325 accessions forming the nucleus of the USDA's wild species sunflower collection. There was no formal structure to the collection at that time.

The USDA-ARS formally established a wild *Helianthus* repository at Bushland, TX, in 1976. The objective of the program was to establish and maintain a wild sunflower germplasm collection containing as many accessions of the known wild species as resources permitted. The decision to create a permanent wild species collection greatly increased the number of plant explorations for wild *Helianthus.*

During 1976, Drs. Charlie Rogers and Tommy Thompson (USDA-ARS, Bushland, TX) undertook an exploration in Texas and New Mexico, adding 200 accessions to the collection. In 1978, they undertook explorations to the western, southwestern, and southeastern USA adding 175 accessions. Several short explorations collecting wild sunflower species were made throughout the USA in 1979 when the USDA-ARS hosted a delegation from the VNIIMK Research Station, Krasnodar, the former USSR.

In 1980, Drs. Gerald Seiler (USDA-ARS, Bushland, TX) and Luka Cuk, Institute of Field and Vegetable Crops (IFVC), Novi Sad, Yugoslavia collected 400 accessions of wild sunflower from the southeastern USA (Seiler and Cuk, 1981; Cuk and Seiler, 1985). In a 1984 exploration to southern Texas, Gerald Seiler collected 32 accessions of annual *H. argophyllus, H. debilis* and *H. praecox*. The most significant addition to the germplasm collection was 20 populations of *H. argophyllus*.

An exploration to the eastern and northeastern USA was undertaken by Drs. Gerald Seiler (USDA-ARS, Bushland, TX), Bill Roath (USDA-ARS, Ames, IA), and Dragon Skoric (IFVC, Novi Sad, Yugoslavia) in 1985. About 100 accessions of wild perennial sunflower were added to the collection. The most significant contribution was the addition of 23 *H. tuberosus* accessions (Seiler et al., 1987).

In 1987, an exploration was made to the Pacific Northwest, USA, by Gerald Seiler (UDSA-ARS, Bushland, TX), Jeff Pomeroy (USDA-ARS, Ames, IA), and Radovan Marinkovic (IFVC, Novi Sad, Yugoslavia) with the addition of 50 wild species accessions. Most of the populations were annual species, but perennial populations of *H. pumilus*, and *H. cusickii* were added to the collection (Seiler et al., 1992a).

An exploration in 1989 to the Great Lakes region of the USA by Gerald Seiler (UDSA-ARS, Fargo, ND), Jeff Pomeroy (USDA-ARS, Ames, IA), Branislav Dozet (IFVC, Novi Sad, Yugoslavia), and Vera Gavrilova [Vavilov Institute (VIR), St. Petersburg, Russia] resulted in the addition of 84 accessions of wild sunflower. Twelve different species were represented, five of which were perennial. This exploration significantly increased the number of perennial *H. giganteus* accessions in the collection. Additional accessions of *H. divaricatus, H. decapetalus*, *H. mollis*, and *H. hirsutus* were also collected (Dozet at al., 1990; Seiler et al., 1990).

In 1991, an exploration to seven Central Great Plains states of the USA by Gerald Seiler (UDSA-ARS, Fargo, ND), S. Duhoon [National Bureau of Plant Genetic Resources (NBPGR) New Delhi, India], Radovan Marinkovic (IFVC, Novi Sad, Yugoslavia), and Cynthia Stauffer (USDA-ARS, Ames, IA), resulted in the addition of 215 accessions representing two annual and six perennial species (Seiler, 1994, 1996a; Seiler et al., 1992b, 1993; Duhoon et al., 1992). Almost half of the accessions were *H. annuus* populations. The most frequently collected perennial was *H. maximiliani*, followed by *H. pauciflorus*, representative of the species distributions in this section of the USA.

The first exploration undertaken outside of the USA was in 1994 to the Prairie Provinces of Manitoba, Saskatchewan, and Alberta, Canada. This collection was undertaken by Gerald Seiler (UDSA-ARS, Fargo, ND) and Mary Brothers (USDA-ARS, Ames, IA), curator of the sunflower collection. Sixty-three accessions of wild sunflower were collected. Thirty-one accessions were annual, while 32 were perennial. Almost 40% of the accessions were *H. annuus*. The collected populations represent the first wild *Helianthus* germplasm from Canada to be incorporated into the USDA sunflower collection. The northern limitation for collection was 53 degrees north latitude. Beyond this latitude the vegetation is predominantly coniferous forests which are not suitable habitat for wild sunflowers (Seiler, 1997; Seiler and Brothers, 1996, 1999).

In 2000, an exploration to southwestern USA (Nevada, Utah, and Arizona) for annual species *H. anomalus* and *H. deserticola* was undertaken by Gerald Seiler (UDSA-ARS, Fargo, ND) and Mary Brothers (USDA-ARS, Ames, IA). All previously identified populations (over 25) of the two species were visited. Both species grow in very specific habitats which are fragile, shifting sand dunes and sandy desert shrub habitat. Due to a drought, only two populations of *H. anomalus* and one *H. deserticola* were available for collection. The addition of these accessions to the collection made seed of these species available for research for the first time in almost 20 years (Seiler and Brothers, 2003).

Exploration for annual serpentine sunflower, *H. exilis,* was undertaken in California by Tom Gulya and Gerald Seiler (both USDA-ARS, Fargo, ND) in 2002 (Seiler and Gulya, 2004). Serpentine sunflower is endemic to serpentine soils and outcrops in California. The distribution of this soil type is the Coastal range and Klamath Mountains, and the western foothills of the Sierra Nevada Mountains. Twenty-six populations were collected during the exploration and added to the wild sunflower collection. This almost tripled the nine populations already in the USDA wild *Helianthus* collection, but did not have adequate seed for distribution. The geographic distribution of this species is much better understood now than 15 years ago when it was considered threatened because of habitat destruction.

In September 2003, an exploration by Tom Gulya and Gerald Seiler (both USDA-ARS, Fargo, ND) was undertaken to California to collect the endemic perennial species *H. californicus*. The species is habitat-specific to riparian areas occurring in both dry and wet sites ranging from small streams to large rivers and is indigenous to central and southern California. The exploration resulted in the collection of 13 accessions of California sunflower. Prior to the exploration, only three accessions were present in the wild sunflower collection.

An exploration to the southeastern USA to collect perennial *H. eggertii*, *H. schweinitizii*, *H. verticillatus* and annual *H. porteri* was made in October 2003 by Tom Gulya and Gerald Seiler (both USDA-ARS, Fargo, ND) and Gary Kong (Queensland Department of Primary

Industries, Toowoomba, Australia). Thirteen populations of *H. eggertii*, and 14 populations of *H. schweinitizii* were collected. Two populations of *H. verticillatus,* a new species for the collection, and eight populations of annual *H. porteri*, also the first accessions of this species were added to the wild species collection. One population of perennial *H. smithii*, a species with a limited distribution was also collected. Two populations of perennial *H. angustifolius* and one of *H. atrorubens* were also collected during this exploration.

Future explorations are planned to help fill the gaps in the current wild species collection. Since the formation of the sunflower germplasm collection in 1976, 15 explorations have occurred (Seiler, 1988; Cuk and Seiler, 1985; Dozet et al., 1990; Seiler, 1987; Seiler et al., 1987; Seiler et al., 1990; Seiler et al., 1992 a, b; Duhoon et al., 1992; Seiler et al., 1993; Seiler and Brothers, 1996, 1999, 2003; Seiler and Gulya, 2004). Multiple researchers traveled the equivalent of several times around the world in search of wild *Helianthus* species. Several explorations were joint efforts between the USDA-ARS and the Food and Agriculture Organization of the United Nations (FAO), European Cooperative Research Network (ESCORENA), Wild Species Working Group, the International Plant Genetic Resources Institute, European Cooperative Program for Genetic Resources (IPGRI/ECP/GR), US Agency for International Development (USAID), USDA Office of International Cooperation and Development (OICD), and the National Bureau of Plant Genetic Resources (NBPGR) New Delhi, India.

The collection efforts have resulted in the assemblage of the USDA-ARS wild species collection that is the most complete collection in the world. It is presently located at the National Plant Germplasm System, Plant Introduction Station at Ames, IA. Currently, the wild *Helianthus* collection contains 2163 accessions, about two-thirds of which are annual species (Brothers and Seiler, 2002; Marek et al., 2004). The germplasm collection contains seeds or rootstocks from populations of all but one species, *H. laciniatus*, and one subspecies *H. niveus* ssp. *niveus*, but lacks sufficient populations of many species to be completely representative of the genetic variability in nature. From 1976 to 1996, 10,000 samples of wild sunflowers have been distributed to 300 researchers in 30 countries. These accessions have become the basis of wild species research programs in Argentina, France, Italy, Spain, Germany, Bulgaria, Romania, Czechoslovakia, Hungary, Russia, Yugoslavia, India, China, and Mexico. Notable is the collection at the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia, which contains 39 of the 50 wild species (IBPGR, 1984; Cuk and Seiler, 1985). The wild species collection of the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, is also notable containing 428 accessions representing 37 of the 50 species of *Helianthus* (Christov et al., 2001). The wild species collection maintained at INRA, Montpellier, France has more than 600 accessions of 45 of the 50 wild sunflower species (Serieys, 1992).

While progress has been made in the collection and preservation of the wild sunflower species, the present germplasm collection contains only a portion of the available genetic variability in *Helianthus*. Additional populations of several species should be collected; particularly those species that are endangered, threatened, or indigenous to habitats where development is threatening.

Collection of germplasm not only serves a valuable purpose in saving germplasm, but it also provides valuable information about the diverse habitats occupied by wild sunflowers and associated species. This information is particularly important for the genus *Helianthus* because of the co-evolution of its species and associated native insect and pests. Knowledge of a particular habitat and adaptations of a species occurring therein can often help to identify potential sources of genes for a desired trait. Based on the habitat of a species and its immediate environment, selection of potential species or population for a particular characteristic may become easier, more accurate, and more efficient.

Species Distribution. The North American species of *Helianthus* are found in virtually all parts of the United States and several species extend into Canada and a few into Mexico. They occupy a variety of habitats. Most species are found in fully open habitats with a few growing in rather dense shade. A number of species can be classified as weeds. *Helianthus annuus* which has the most extensive distribution of any species apparently grows only in areas disturbed by man. At least one subspecies, a densely pubescent form of *H. nuttallii* ssp. *parshii* has become extinct as the result of man's activities. Other species have suffered because of habitat destruction, restricting their distribution and very existence.

The annual species of *Helianthus* are generally distributed in central-western USA, and the Gulf of Mexico Coast, the exception being *H. annuus* which is widespread from Canada to Mexico, and coast-to-coast. The perennial species of the section Ciliares are confined to the west. The perennial species of the series Pumili are found in the Rocky Mountains, the northwest, and California, whereas the perennial species of the series Ciliares are found in the southwest and Mexico. The perennial species of the section Divaricati are concentrated in the Appalachian Mountains of the eastern USA with secondary centers in the Ozark Mountains of eastern USA, and Florida. Two perennial species of this section, *H. tuberosus* and *H. pauciflorus* extend west to the Central Great Plains of the USA. The perennial species of the series Gigantei have three Appalachian representatives, *H. giganteus, H. resinosus*, and *H. schweinitizii*. All perennial species of the Microcephali series are centered in the Appalachians, and the members of the Angustifolii series are perhaps best considered Floridian. The series Atrorubentes has species in all three eastern centers, Floridian, Ozarkian, and Appalachian. More detailed information about the distribution of *Helianthus* species can be found in Heiser et al. (1969) and Rogers et al. (1982).

The occurrence of *Helianthus* in Mexico has been recently documented by Gomez-Sanchez and Gonzalez (1991). They collected 12 species of *Helianthus* from Baja California, Baja California Sur, Sonora, Sinaloa, Chihuahua, Durango, Coahulia, Nuevo Leon, Tamaulipas and Zacatecas, Mexico. Scoggan (1978) lists 13 species of *Helianthus* occurring in Canada. They are distributed primarily in the Prairie Provinces and northward to edges of the boreal forests.

Species of *Helianthus* have become naturalized weeds and garden plants worldwide. The most common is Jerusalem artichoke, *H. tuberous*, which has rapidly spread worldwide. Konvalinkova (2003) indicated that it was introduced to Europe as a crop and ornamental at the beginning of the 17th century and since then has become invasive, spreading quickly into central Europe. Dozet et al. (1993) described populations of *H. tuberosus* collected in Montenegro, of the former Yugoslavia.

Two wild annual species of *Helianthus*, *H. annuus* and *H. petiolaris* were accidentally introduced into Argentina, probably more than 50 years ago (Poverene at al., 2002, 2003, 2004). These species grow as weeds in seven provinces and overlap about 50% of the sunflower production areas in Argentina. Intraspecific hybrids have been observed for many years (Covas and Vargas López, 1970; Ferreira, 1980).

Three wild species of *Helianthus*, *H. annuus, H. tuberosus*, and *H. ciliaris* have been reported to occur in the flora of Australia (Harden, 1992). *Helianthus annuus* is listed as widespread along roadsides and disturbed sites, while *H. ciliaris* is listed as a weed of cultivation and roadsides, and *H. tuberosus* is listed as a minor weed along roadsides and in wasteland near habitation.

Along the coast of the Inhanbane Bay, Mozambique, two wild sunflower species, *H. argophyllus*, and *H. debilis* ssp. *cucumerifolius* grow along the seashore (Olivieri at al., 1999; Vischi et al., 2002). Since *Helianthus* is native to North America it is suspected that the wild species were introduced into Africa through the slave trade route between Africa and coastal Texas (Capela and Medeiros, 1987). Preliminary results of Vischi et al. (2003) showed that whatever was the manner of colonization of wild sunflowers in Africa, they appear to be subpopulations of the Native American species.

Locating Populations of Helianthus Species. The genus *Helianthus* is an extremely diverse group of species whose geographic distribution ranges from nearly universal in all the continental United States, to species which are found only in one state, to those species found only in a few isolated areas within a single US state. Similarly, the species' habitats may range from a generalized prairie habitat, with little preference for soil type or moisture regime, to a specific, well-defined environment such as active sand dunes. The two best references describing the distribution and habitats for all *Helianthus* species are the Heiser et al. (1969) monograph of the genus *Helianthus* and the work by Rogers et al. (1982), which has excellent maps of the United States showing the distribution of each species. These two sources document the occurrences of each species, accurate to their publication date, and give a brief description of the species' habitats.

For more specific information on the location of individual species, one might consult regional, state or local flora guides, but these are generally not specific enough. Thus, for example, in *Handbook of North Dakota Plants* by Stevens (1963) lists the distribution of *H. tuberosus* as "along streams or other low ground, throughout the state, but chiefly in the eastern part." Some more recent floras are more specific, such as the *Flora of the Great Plains*, which covers 28 *Helianthus* species (Great Plains Flora Association, 1986). Thus for a geographically limited species like *H. salicifolius*, this flora points the reader to limestone prairies in western Missouri, the eastern quarter of Kansas and adjacent Oklahoma. Some floras have an accompanying atlas to pictorially depict the geographic range of plants. The *Atlas of the Flora of the Great Plains* has maps with county outlines by which the reader can see exactly in which of the four Oklahoma counties, six Missouri counties, and 34 counties in Kansas that *H. salicifolius* is documented to occur (Great Plains Flora Association, 1977). Botanists in several states are now opting to make this information available electronically on the internet so it can be updated easily rather than publishing a book which is costly and more difficult to revise. Thus, for the county-by-county distribution of *Helianthus* specimens in Florida one could consult http://www.plantatlas.usf.edu/default.asp, while to find information on *Helianthus* distribution in Tennessee counties, one could consult http://tenn.bio.utk.edu/ vascular/vascular.html. Not all states have either a printed or an on-line vascular plant atlas, but those that have working websites (or in progress) are listed in Table 3.

Local Floras. Many states in the United States have had extensive investigations made of the local flora, generally by professional botanists or graduate students. The information in these "local floras," which generally cover a county, a state park, or a very limited area, is generally much more specific than in state floras. Thus the location of a population of a given species may be pinpointed to a specific trail in a state park. Table 4 contains a partial listing of local floras for the state of California, which has one of the more thoroughly characterized state floras, as an example of the local floras that should be consulted.

Table 3. A partial listing of the internet addresses of herbaria in the United States, some of which have database access via the internet for plant location information.

ARKANSAS: University of Arkansas Herbarium (UARK). Director: John Gentry, 501-575-4372, gentry@comp.uark.edu

ARIZONA: Arizona State University Plant Herbarium. Leslie R. Landrum, Curator, Phone: (480) 965-6162 http://lifesciences.asu.edu/herbarium/

CALIFORNIA: CalFlora Database Project. http://www.calflora.org/. Note: fee charged for access.

COLORADO: University of Colorado Herbarium. Dr. Mark Simmons, Curator psimmons@lamar.colostate.edu. http://herbarium.biology.colostate.edu/database.htm

FLORIDA: Wunderlin, R. P., and B. F. Hansen. 2003. Atlas of Florida Vascular Plants Institute for Systematic Botany, University of South Florida, Tampa. (http://www.plantatlas.usf.edu).

GEORGIA: The Vascular Plant Distribution Atlas of Georgia (in preparation). http://www.plantbio.uga.edu/herbarium/herbarium/atlas.htm

IDAHO: University of Idaho Stillinger Herbarium. Pam Brunsfeld: pambruns@uidaho.edu. http://www.sci.uidaho.edu/biosci/herbarium/database.asp

MISSOURI: Weber, W.R., W. T. Corcoran, P. L. Redfearn, and M. S. Brunell. Atlas of Missouri Vascular Plants http://biology.smsu.edu/Herbarium/atlas/atlas_of_missouri_vascular_plant.htm)

NEW ENGLAND: Angelo, R. and D.E. Boufford. 2003. Atlas of the Flora of New England (in progress). http://neatlas.huh.harvard.edu/

NEVADA: Nevada Natural Heritage Program. Nevada Rare Plant Atlas. 2001. http://heritage.nv.gov/atlas/atlasndx.htm

OKLAHOMA: Hoagland B.W., Buthod A.K., Butler, I.H., Crawford, P.H.C., Udasi, A.H., Elisens, W.J., and Tyrl, R.J. 2004. Oklahoma Vascular Plants Database. http://geo.ou.edu/botanical or http://www.biosurvey.ou.edu/atlasdesc.htm

OREGON: Oregon Plant Atlas Project: Atlas of Vascular Plant Distributions (in preparation). http://www.oregonflora.org/OFP/atlas.htm

SOUTH CAROLINA: Nelson, John. 2000. South Carolina Plant Atlas. http://cricket.biol.sc.edu/herb/

TEXAS: Texas A&M University Bioinformatics Working Group. Vascular Plants Endemic to Texas. http://www.csdl.tamu.edu/FLORA/endemics/endemae.htm

UTAH: Albee, B. J., L. M. Shultz and S. Goodrich. 2004. Atlas of the Vascular Plants of Utah. http://www.nr.usu.edu/Geography-Department/utgeog/utvatlas/ut-vascatlas.html

WASHINGTON: University of Washington Herbarium (WTU) e-mail: wtu@u.washington.edu. http://depts.washington.edu/wtu/home.htm

WISCONSIN: Cochrane, T. and M. Wetter. WISFLORA: WISCONSIN VASCULAR PLANTSPECIES.
http://www.botany.wisc.edu/wisflora/scripts/SearchResults.asp?Genus=Helianthus

Table 4. A partial listing of the local floras documenting plant distribution and occurrence in specific areas of the state of California.

A flora of San Diego County, California. Sweetwater River Press, National City, CA. 241 p. (Beauchamp, R. M. 1986).

A flora of Sonoma County. CA Native Plant Society. 347 p. (Best, C., J. T. Howell, W. Knight, I. Knight, and M. Wells. 1996).

The flowering plants and ferns of Mount Diablo, California. Gillick Press, Berkeley CA. 290 p. (Bowermann, M. L. 1944).

The rare and endangered plants of San Mateo and Santa Clara County. Monocot Press. Half Moon Bay, CA. 139 p. (Corelli, T. and Z. Chandik. 1995).

Flowering plants of the Santa Monica Mountains, coastal and chaparral regions of southern California. Capra Press. 239 p. (Dale, N. 1986).

Annotated checklist of the East Bay Flora. Spec. Pub. #3 of Ca. Native Plant Society East Bay Chapter in assoc. with the Univ. & Jepson Herbaria. 114 p. (Ertter, B. 1977).

Common riparian plants of California. Pickleweed Press. 140 p. (Faber, P. M. and R. F. Holland, 1988).

A flora of Lassen Volcanic National Park, California. Wasmann J. Biology (Univ. San Francisco) 19:1-185. (Gillett, G. W., J. T. Howell, and H. Leschke. 1961).

Plants of the Tahoe Basin. U. Cal. Press, Berkeley. 308 p. (Graf, M. 1999).

The vascular plants of San Luis Obispo Cunty, California. U. Cal. Press, Berkeley. 350 p. (Hoover, R. F. 1970).

Manual of the flowering plants and ferns of Marin County, California. U. Cal. Press, Berkeley. 323 p. (Howell, J. T. 1949).

A flora of San Francisco, California. Wasmann J. Biology (Univ. San Francisco) 16:1-157 (Howell, J. T., P. H. Raven and P. Rubtzoff. 1958).

An illustrated field key to the flowering plants of Monterey County. CA Native Plant Society. 401 p. (Matthew, M. A. 1997).

Manual of the vascular plants of Butte County, California. CA Native Plant Society. 349 p. (Oswald, V. H. and L. Ahart. 1994).

A flora of the vascular plants of Mendocino County, California. Wasmann J. Biology (Univ. San Francisco) 48/48:1-387. (Smith, G. L. and C. R. Wheeler. 1990-1).

Flora of the Santa Cruz Mountains of California. Stanford Univ. Press. 434 p. (Thomas, J. H. 1961).

***Herbaria Information*.** Many herbaria have inventoried their holdings and have this information in databases that are web-accessible. Thus, one can access the specific information from each herbarium specimen of a particular species. While many older specimens have very general location information, newer specimens may have GPS coordinates in addition to text descriptions of the collection site. For example, by accessing the Colorado State University herbarium website for information on *Helianthus pumilus*, we first discover that they have 40 specimens on hand, collected from 1897 to 2000. Specimen #80547, collected on 13 June 2000, was found at GPS coordinates 40.6950 N, 105.2833 W., on the Greyrock trail, west of Ft. Collins off highway 14. While many herbaria include GPS coordinates, many times these are retroactively determined based on the verbal location description, so the accuracy of the GPS coordinates may range up to several kilometers from the actual site. Another consideration when using herbaria specimen data is the age of the collection. With the impact of "civilization," many plant sites even a decade old no longer exist. Listings of state herbaria are found in Table 3. Some, but not all of these, have specimen databases accessible through the internet, while for others, one would need to contact the herbarium staff to request such information.

Rare Species. *Helianthus* species which are classified either on federal or state lists as rare, endangered, or threatened are often easier to locate than species regarded as common, because the rarer species are often monitored by various state and federal agencies. Thus, the serpentine sunflower (*H. exilis*), once classified by the state of California as rare, was monitored by both the California Fish & Wildlife Service (CaFWS) and by the National Forest Service. Forest service rangers and contract botanists recorded the locations of *H. exilis* populations in the National Forests in northern CA in the 1980s and their multiple page reports are on file in the CaFWS offices in Sacramento. *Helianthus eggertii*, federally classified as a "threatened species" is monitored by both the U.S. Fish and Wildlife Service and the Tennessee Natural Heritage Program (White and Ratzlaff, 1999), and there is detailed information on at least 128 populations within Tennessee. Similarly, *H. schweinitzii*, classified as "endangered" in its limited range in southwestern North Carolina, is monitored by the North Carolina Natural Heritage Program, and detailed information is available for the 38 or so known populations.

Local Botanists. The veracity of the above plant location information is all dependent upon changes that may have occurred since the initial observation. In the intervening time period, whether it is a year or a decade, events such as a flash flood, a drought, road construction or forest encroachment will impact not only the health of a plant population, but also its existence. Thus, the best source of information on the location of plant populations is firsthand contact with local botanists, either by phone, e-mail or mail. Local botanists can be professionals or dedicated amateurs affiliated with universities, with government agencies like the National Forest Service (NFS) or the Bureau of Land Management (BLM), with state agencies like state parks or departments of natural resources, or with private groups like the Nature Conservancy or various state "Native Plant Societies." Some of our most successful collecting have been when a local botanist offers to accompany us for a period of time to collect seed. The accompanying botanist also is invaluable in helping us to identify other local flora and to understand the interaction of local climate and geology with the *Helianthus* taxa we are seeking. Some of the botanists have also offered to collect seed from populations that were not mature during our visit, and to accompany us on future collection trips.

Funding. Most recent explorations to collect wild *Helianthus* seeds have been funded by the USDA-ARS's Plant Germplasm Exploration Office, which is part of the USDA National Germplasm Resources Laboratory (http://www.barc.usda.gov/psi/ngrl/about.htm) located in Beltsville, MD. Detailed trip proposals of a specified format are submitted for peer review by June for the subsequent fiscal year (October 1 to September 30), so trips are planned at least a year in advance. Explorations usually have two to three USDA personnel. On several previous explorations, researchers from other countries have also participated, with funding from their institutions and governments.

Trip Protocol. Plant exploration trips generally last from 10 to 14 days. Since collection sites are distant from Fargo, ND, and Ames, IA, we fly to the collecting area and rent a vehicle. The first day of the trip consists of reaching the location and sometimes contacting local botanists for last-minute information. During the 8 to 12 days actually spent collecting, we generally drive about 250 miles daily, mostly on secondary and sometimes unpaved roads, which in itself takes anywhere from five to seven hours traveling from site to site. Once we arrive in the general location of a presumed *Helianthus* site, we often must hike some distance to locate the population. In many state parks, vehicle travel on dirt roads is limited to official vehicles. After locating a population of *Helianthus*, our first objective is to determine whether there are enough plants with mature seed to harvest, and if not, we simply take notes on the population. Our seed collection strategy is to harvest from as many plants as possible (to maximize genetic diversity) and to collect multiple heads per plant (to achieve in excess of the 2000 seed threshold set by the NCRPIS for distribution). Heads with their ray petals fully wilted are considered to have mature seed, despite their high moisture content. More mature heads, with drier seed, may have already begun to shatter (dehisce) their seeds, and thus seed recovery may be less than with less mature heads. Seeds are placed in paper bags, labeled with the site number, and the tops left open to facilitate drying during the trip. Extensive site information is recorded for each population, starting with date, GPS coordinates, and elevation. Other information includes general habitat and associated plants, soil type, exposure and slope, and a text description of the directions to the site. Population characteristics, such as number of plants, area covered, and phenological stage are recorded, as well as any observations on disease, predation, or other threats to the *Helianthus* population or site.

The number of accessions and volume of seed collected varies widely from trip to trip. The most recent trip in 2003 to the southeastern USA, for example, netted up to 80,000 seeds of *H. porteri* per population at some sites. Looking at the most recent three collection trips, which resulted in 81 collections of eight species, only 13 of the collections netted fewer than 2,000 seeds, so 84% of our new collections have enough seed for immediate distribution. The number of seeds collected obviously depends upon the species, our timing relative to flowering, and the size of the individual populations. With some species, such as *H. schweinitzii* and *H. porteri*, we were very successful and collected an average of 32,000 and 59,000 seeds per site for the two species, respectively. Other species, such as *H. californicus* whose population sizes ranged from a single plant to about 150, netted only an average of 2,400 seeds/collection, with six of our 13 collections having fewer than 2,000 seeds. In general, we have made an average of three collections per day, with a high of seven, but this is not counting the other sites visited each day, from which for whatever reason we were not able to collect seed. During each trip there is often a day or several partial days where we must travel between widely separated distribution areas of a particular species, which also decreases the “success rate” of collections per day.

Generally we will ship seed heads back to Fargo midway through the trip, using an express courier, and carry the last collections with us. Upon arrival in Fargo, the bags containing the seeds will be placed in a drier set no higher than 32C for several days. Because of the small to very small seed size, all seed cleaning is initially done by hand. The seed heads are gently broken and then sieved over various sized screens to remove the large chaff. The remaining seed is then cleaned using a blower with a varying amount of air. The cleaned seed is then prepared for long-term storage at 4C and 35% humidity.

Before the seed is sent to the NCRPIS for cataloguing and storage, we generally conduct several laboratory and greenhouse evaluations. Germination is assessed using various methods to overcome dormancy (Chandler and Jan, 1985; Seiler, 1993, 1996b, 1998; Brothers et al., 2000), so that the number of viable seeds can be determined. One hundred-seed weights are obtained and the oil content and fatty acid composition determined. With the 100-seed weight determined, we can calculate the total number of seeds collected to assess the trip success.

In addition to the laboratory tests of the seed, we also strive to do some preliminary disease resistance evaluations on the newly collected species. The germinated seedlings from the viability test are inoculated with a mixture of virulent downy mildew [*Plasmopara halstedii* (Farl.) Berl. and de Toni] races, a test which takes only two weeks to complete (Gulya, 1996). Other seedlings are transplanted into pots for a similar evaluation for resistance to rust caused by *Puccinia helianthi* (Gulya and Masirevic, 1996). In some instances we will also test for resistance to sunflower mosaic virus or rust caused by *Coleosporium helianthi* (Schwein.) Arth., all of which can be done on seedlings in greenhouse tests.

Future Collecting Trips. The sunflower seed collection, housed at the USDA-North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, currently has seed of all but two taxa of the 65 *Helianthus* taxa (species and subspecies) (Marek et al., 2004). The two missing taxa, *H. niveus* ssp. *niveus* and *H. laciniatus*, are exclusively Mexican in distribution. The USDA seed collection of wild *Helianthus* species makes the sunflower collection one of the best among crop species in the UDSA Plant Introduction system. The extensiveness of the collection is, however, misleading. The utility of a germplasm collection is dependent upon the genetic diversity of each species (i.e., the number of accessions per species) and the availability of seed for researchers. Of the 65 *Helianthus* taxa, currently 23 are classified as "unavailable" as the seed quantity is below the 2,000 seed threshold limit, below which distribution is stopped pending either seed regeneration or recollection (Tables 5 and 6). Additionally, there are another 14 taxa that have three or fewer accessions. Thus, 37 of the 65 taxa are either not available or have few accessions and may not be representative of the genetic diversity of that species. Of these 37 taxa, 29 are perennial species, primarily from the southwest and southeastern US. Some perennial *Helianthus* species are time-consuming or impractical to regenerate for seed increases at the NCRPIS in Iowa or at the "satellite" station in Parlier, CA, and thus the best solution for many of the 37 taxa is continued plant exploration trips to collect seeds.

Table 5. A listing of the annual *Helianthus* species and subspecies, and the total number of accessions in the USDA-NCRPIS collection at Ames, Iowa, and the number available for distribution.

	Annual Species	**Total**	**Available**
1	*H. agrestis*	5	0
2	*H. annuus*	1006	798
3	*H. anomalus*	12	2
4	*H. argophyllus*	48	8
5	*H. bolanderi*	8	0
6	*H. debilis* ssp. *cucumerifolius*	7	7
7	*H. debils* ssp. *debilis*	26	12
8	*H. debilis* ssp. *silvestris*	22	22
9	*H. debilis* ssp. *tardiflorus*	5	5

Table 5. (Continued)			
10	*H. debilis* ssp. *vestitus*	3	3
11	*H. deserticola*	12	1
12	*H. exilis*	36	20
13	*H. neglectus*	28	28
14	*H. niveus* ssp. *niveus*	0	0
15	*H. niveus* ssp. *tephrodes*	2	0
16	*H. paradoxus*	10	0
17	*H. petiolaris* ssp. *canescens*	16	13
18	*H. petiolaris* ssp. *fallax*	31	29
19	*H. petiolaris* ssp. *petiolaris*	108	97
20	*H. porteri*	8	8
21	*H. praecox* ssp. *hirtus*	8	7
22	*H. praecox* ssp. *praecox*	8	8
23	*H. praecox* ssp. *runyonii*	24	24

Table 6. A listing of the perennial *Helianthus* species and subspecies, and the total number of accessions in the USDA-NCRPIS collection at Ames, Iowa, and the number available for distribution.

	Perennial Species	**Total**	**Available**
1	*H. angustifolius*	21	2
2	*H. arizonensis*	2	0
3	*H. atrorubens*	19	3
4	*H. californicus*	16	6
5	*H. carnosus*	1	0
6	*H. ciliaris*	6	1
7	*H. cusickii*	4	0
8	*H. decapetalus*	33	17
9	*H. divaricatus*	42	7
10	*H. eggertii*	15	10
11	*H. floridanus*	5	0
12	*H. giganteus*	34	2
13	*H. glaucophyllus*	2	0
14	*H. grosseserratus*	48	11
15	*H. gracilentus*	6	0
16	*H. heterophyllus*	9	0
17	*H. hirsutus*	22	0
18	*H. laciniatus*	0	0
19	*H.* x *laetiflorus*	12	1
20	*H. laevigatus*	8	0
21	*H. longifolius*	1	0
22	*H. maximiliani*	80	37
23	*H. microcephalus*	15	0
24	*H. mollis*	29	8
25	*H.* x *multiflorus*	1	0

Table 6. (Continued)			
26	*H. nuttallii* ssp. *nuttallii*	22	13
27	*H. nuttallii* ssp. *rydbergii*	12	12
28	*H. occidentalis* ssp. *occidentalis*	2	1
29	*H. occidentalis* ssp. *plantagineus*	14	3
30	*H. pauciflorus* ssp. *pauciflorus*	13	0
31	*H. pauciflorus* ssp. *subrhomboideus*	19	5
32	*H. pumilus*	6	1
33	*H. radula*	18	0
34	*H. resinosus*	10	0
35	*H. salicifolius*	3	0
36	*H. schweinitzii*	15	14
37	*H. silphioides*	5	0
38	*H. simulans*	4	1
39	*H. smithii*	2	1
40	*H. strumosus*	42	8
41	*H. tuberosus*	116	5
42	*H. verticillatus*	2	2

The 36 *Helianthus* species requiring seed collection can be grouped into nine geographic areas; eight within the U.S. and one in the Mexican states of Sonora and Baja California (Table 7). Most of these nine areas contain multiple taxa of interest, ranging up to eight in Florida and nine in the southeastern USA areas. In addition, all of these areas contain other *Helianthus* species of lesser priority because their seed numbers in the collection are adequate. For example, both North Carolina and Georgia have at least 22 *Helianthus* species (Rogers et al., 1982). Thus, a collecting trip may net seeds of species other than the targeted taxa. It may also be wise to consider collecting seeds of near relatives of *Helianthus* (e.g., *Viguiera* spp., *Phoebanthus* spp.) for the possibility of future intergeneric crosses with *Helianthus*.

Assuming one collecting trip per year, it may be possible to collect seed of all remaining 36 taxa within the next decade. One complicating factor is that not all species within a given area have the same flowering period. These situations may require two collecting trips, preferably within the same calendar year if non-mature populations of a late-blooming species are observed during the first collecting trip. Also based upon our experience, finding large populations of plants with mature seeds entails a degree of chance, and in some instances a second trip maybe needed to collect a given species' seeds.

Table 7. *Helianthus* species underrepresented in the USDA seed collection, grouped by geographic distribution.

	Area and Species	Total	Available	Flowering	Habitat	Range
1	**Mexico**					
	niveus ssp. *niveus**	0	0	All year	sand dunes	Baja, Mexico
	niveus ssp. *tephrodes**	2	0	Sept. to May	sand dunes	CA; Sonora, Mexico
	gracilentus	6	0	May to Oct.	dry slopes	CA; Baja, Mexico
	laciniatus	0	0	June to Sept.	wet, alkaline	NM, Mexico
2	**Florida**					
	*agrestis**	5	0	Oct. to Dec.	damp muck	FL, GA
	carnosus	1	0	June to Sept.	wet sand	FL
	debilis ssp. *vestitus**	3	3	March to Sept.	sand	FL
	floridanus	5	0	Sept. to Oct.	sandy	FL, GA, SC
	heterophyllus	9	0	Aug. to Oct.	wet sand	FL, LA, NC
	longifolius	1	0	Sept. to Oct.	variable	GA, AL
	radula	18	0	Sept. to Nov.	wet sand	FL, GA, SC
	simulans	4	1	Sept. to Nov.	variable	FL, LA
3	**West Texas**					
	ciliaris	6	1	June to Oct.	variable	TX, NM
	laciniatus	0	0	June to Sept.	wet, alkaline	NM, Mexico
	*paradoxus**	10	0	Sept. to Oct.	wet	TX, NM
4	**Pacific NW**					
	*bolanderi**	8	0	July to Sept.	valleys	CA, OR
	cusickii	4	0	April to Aug.	rocky hillsides	CA, OR, NV, WA
5	**S to Central Midwest**					
	occidentalis ssp. *occidentalis*	2	1	July to Sept.	dry sand	MO, IL, WI
	occidentalis ssp. plantagineus	14	3	July to Sept.	variable	TX, AR
	pauciflorus ssp. *pauciflorus*	13	0	Aug. to Sept.	variable	KS, MO, IA, WI
	salicifolius	3	0	Aug. to Sept.	alkaline soils	KS, MO, OK
	silphioides	5	0	Aug. to Sept.	variable	AR, TN,MO,
6	**Desert Southwest**					
	*anomalus**	12	2	May to Oct.	dry sand	UT, AZ
	arizonensis	2	0	June to Aug.	light soil/ sand	AZ, NM
	*deserticola**	12	1	May to Oct.	sand	NV, UT
7	**Southeast**					
	angustifolius	21	2	Sept. to Oct.	swampy	SOUTHEAST
	atrorubens	19	3	Aug. to Sept.	variable	NC, TN, SC, VA
	glaucophyllus	2	0	Aug. to Sept.	shady	TN, NC
	hirsutus	22	0	July to Oct.	dry, open	SOUTHEAST
	x *laetiflorus*	12	1	July to Oct.	prairie	TN, NC
	laevigatus	8	0	Aug. to Sept.	shale barrens	NC, VA
	microcephalus	15	0	Aug. to Sept.	variable	TN, KY, NC, SC
	resinosus	10	0	July to Sept.	variable	AL, GA, NC, SC
	smithii	2	1	Aug. to Sept.	shale barrens	AL, GA, NC, SC
8	**Rocky Mountains**					
	pumilus	6	1	July to Sept.	rocky soil	CO,WY
9	**Northeast**					
	giganteus	34	2	Aug. to Oct.	wet	NORTHEAST

Annals are denoted by an asterisk * after the species.

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Conclusions

Wild *Helianthus* species have been and continue to be an invaluable source of new genes for the improvement of cultivated sunflower. Over the past several decades genes for resistance to several diseases, such as rust, downy mildew, powdery mildew, broomrape, Sclerotinia head rot, Sclerotinia stalk rot, and resistance to insects such as sunflower moth have been identified from the wild *Helianthus* species and successfully transferred into cultivated sunflower. Other useful traits found in wild *Helianthus* species include cytoplasmic male sterility, altered fatty acid composition, salt tolerance and herbicide resistance. The majority of these gene transfers have been from the annual *Helianthus* species, which comprise only a third of the species of the genus. Exploiting the genetic diversity of the wild perennial *Helianthus* species will present more of a challenge than the annual species for a number of reasons. First, many perennial *Helianthus* species are poorly represented in the USDA's seed collection, thus necessitating further exploration to make seed available for research. Secondly, many perennial species have different polyploidy levels, which complicate the production of fertile seed in crosses with diploid cultivated sunflower. Lastly, since perennials usually do not flower the first year, this delays the speed with which interspecific crosses can be made. Despite these challenges, the perennial members of *Helianthus* represent an untapped potential for new genes for the improvement of cultivated sunflower. Their collection and preservation will ensure the availability of seed for future researchers and will also aid in conservation efforts to ensure the preservation of the rarer *Helianthus* species in threatened habitats. The sunflower research community has a unique opportunity to collect, preserve, and utilize genetic resources of the wild relatives of *Helianthus* which other crops do not have, owing to the diversity of the wild species present within our own country's borders. We hope that one legacy this generation of scientists passes on to future scientists is a diverse collection of wild *Helianthus* species which can be used to improve cultivated sunflower in the future.

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