Evaluation of wild annual *Helianthus* species for resistance to downy mildew and Sclerotinia stalk rot

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Abstract

Resistance to downy mildew and Sclerotinia stalk rot in wild annual *Helianthus* species was evaluated in greenhouse studies. Resistance to three downy mildew of increasing virulence was progressively less prevalent in a study encompassing 286 accessions of wild *H. annuus*. Resistance to the most virulent race (773) was, however, noted in 26 accessions which all had >90% plants immune to downy mildew. In a second study dealing with 17 annual *Helianthus* species other than *H. annuus*, resistance to a mixture of downy mildew races (with a composite phenotype of 777) was found in 15 of the 17 species examined. *Helianthus argophyllus* and *H. debilis ssp. debilis a*nd ssp. *cucumerfolius* were the species with the most resistant accessions. Twelve accessions had >90% plants immune to the 777 downy mildew mixture, and two accessions had all plants immune. In a third study, resistance to Sclerotinia stalk rot in two annual *Helianthus* species was examined in a greenhouse trial. Accessions of *H. exilis*, the serpentine sunflower endemic to California, ranged from 100% resistant to 100% susceptible, while *H. porterii*, endemic to granite outcrops in southeastern U.S., also had highly resistant accessions. The resistant accessions will be tested in inoculated field trials for verification.

Introduction

The genus *Helianthus* is composed of 14 annual and 37 perennial species. Wild *Helianthus annuus* has been a valuable source of disease resistance genes, having provided resistance to downy mildew, rust, and Verticillium wilt. Relatively few other *Helianthus* species have been evaluated extensively for disease resistance. Downy mildew (Figures 1 & 2) is a widespread disease of sunflower which can easily be controlled by single dominant genes. *Sclerotinia sclerotiorum*, a widespread, soilborne fungus, causes both a head rot and a stalk rot of sunflower. Resistance to these two *Sclerotinia*-caused diseases are controlled polygenically by different sets of gene. This paper summarizes results from three greenhouse studies which examined (1) the prevalence of resistance to different races of downy mildew (*Plasmompara halstedii*) in several hundred wild *H. annuus* populations, (2) the frequency of resistance to the most virulent races of downy mildew in annual *Helianthus* species other than *H. annuus*, and (3) the levels of Sclerotinia stalk rot resistance in two recently collected annual species: *H. exilis* and *H. porterii*.

Materials and Methods

STUDY 1: Downy Mildew Resistance Screening in Wild *H. annuus*.
Seeds of 286 accessions of wild *H. annuus* were obtained from the USDA-ARS, North Central Regional Plant Introduction Station (NCRPIS), Ames, IA. Forty seedlings of each accession were inoculated with three races of downy mildew (*Plasmopara halstedii*) of increasing virulence in three separate experiments. The races were 300 (moderate virulence), 730 (high), and 773 (extremely high). The experiments were carried out in the greenhouse using standard seedling inoculation methods, and evaluated 12 days after inoculation.

STUDY 2: Downy Mildew Resistance Screening in Other Annual *Helianthus* Species.
Seeds of 291 accessions of 17 annual *Helianthus* taxa were obtained from the USDA-ARS-NCRPIS. The taxa evaluated included: *H. anomalus, H. argophyllus, H. debilis* (subspecies *debilis, cucumerfolius, silvestis, tardiflorus* and *vestitus*), *H. deserticola, H. neglectus, H. niveus* (subspecies *canescens* and *tephrodes), H. petiolaris* (subspecies *fallax* and *petiolaris*), *H. porterii*, and *H. praecox* (subspecies *hirtus, praecox* and *runyonii*). Forty seedlings of each accession were inoculated with a mixture of three downy mildew (DM) races which produced a virulence formula of '777,' which is the most virulent combination of races that currently exist. Inoculated seedlings were grown in the greenhouse and evaluated at 12 days and 18 days after planting, to allow sufficient time for symptom expression on slow growing species.

STUDY 3: Sclerotinia Stalk Rot Resistance Screening of Two Annual *Helianthus* Species.
Recently collected accessions of *Helianthus exilis* (serpentine sunflower) and *H. porterii* (Confederate Daisy) were evaluated in preliminary greenhouse trials in the winter of 2004/5. *Helianthus exilis* is found only in northern California, in seasonally wet habitats in nearly toxic serpentine soils, while *H. porterii* is found only on granite outcrops in the Piedmont area of Alabama, Georgia and South Carolina. From 10 to 20 plants of each accession were grown in pots for 6 to 8 weeks, inoculated with *Sclerotinia* grown on millet, and observed for up to eight weeks for symptoms of wilt. This is part of an ongoing project to identify species that may have stalk rot resistance. An oilseed sunflower hybrid, Carill 270, was used as a susceptible check.

Results

STUDY 1: Downy Mildew Resistance Screening in Wild *H. annuus.*
For the reader unfamiliar with the symptoms of sunflower downy mildew, please refer to Figures 1 and 2. Resistance to each of the three DM races was prevalent in the 286 accessions of wild *H. annuus* (Figs. 3,4,5). As expected, the frequency of resistance decreased with the increasing virulence of each race. With race 300, (the least virulent race used) 31% of all plants tested were susceptible, compared to 50% with race 730 and 63% with race 773. Accessions with 100% resistant plants were found for all three races. When tested with the most virulent race 773, there were 26 accessions (of 286 tested) which had 10% or less susceptible plants. Of these 26 accessions, 20 accessions were collected from Texas; the remaining six highly-resistant accessions were collected in Oklahoma, Kansas, Missouri and New Mexico. This suggests that DM resistance is most frequent in wild *H. annuus* originating in south central U.S., and specifically in Texas (Table 1).

Figure 1. Sunflower seedlings affected by downy mildew, displaying typical chlorosis in leaves colonized by fungus.

Figure 2. Downy mildew affected sunflower plant showing extreme stunting

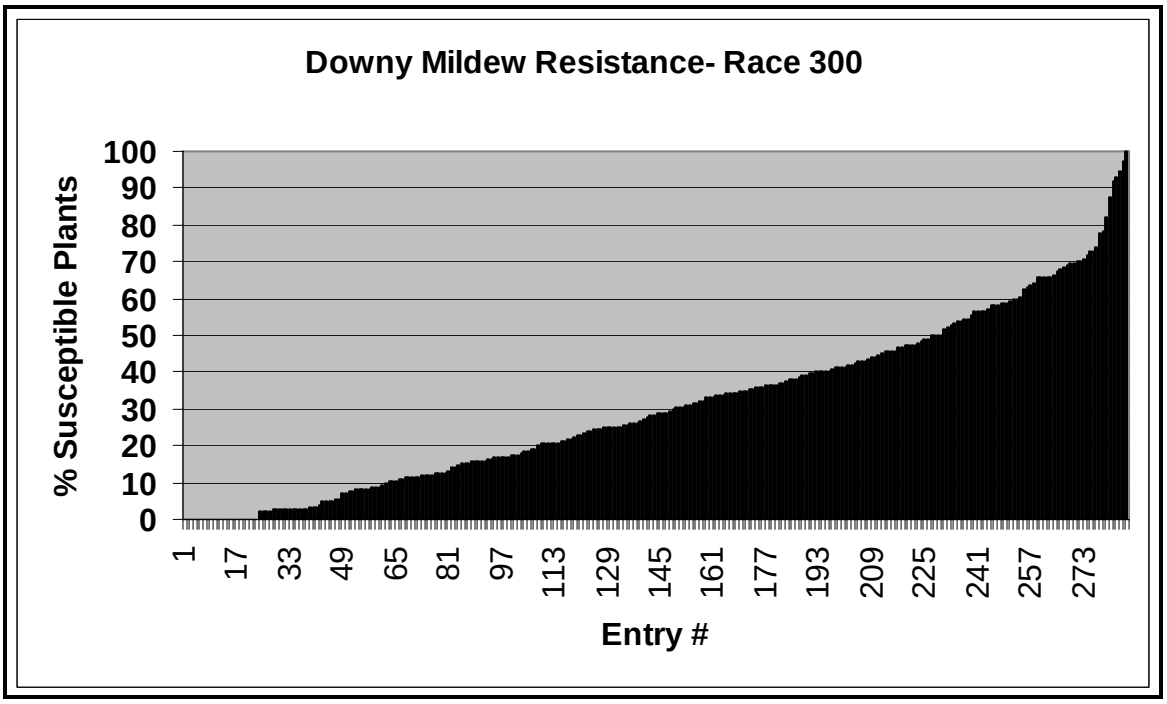

Figure 3. Histogram showing the percentage of wild *Helianthus annuus* plants susceptible to downy mildew race 300 in a test of 286 accessions.

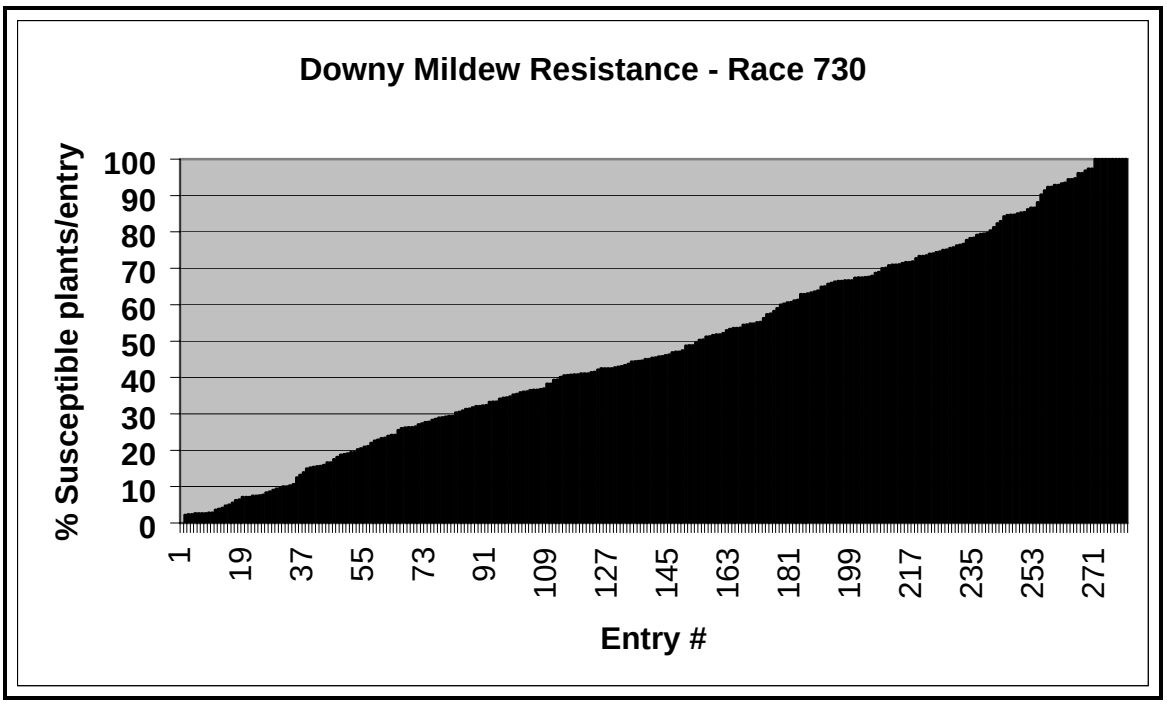

Figure 4. Histogram showing the percentage of wild *Helianthus annuus* plants susceptible to downy mildew race 730 in a test of 286 accessions. Entries are numbered sequentially, and thus entry identification is different in figures 3, 4 and 5.

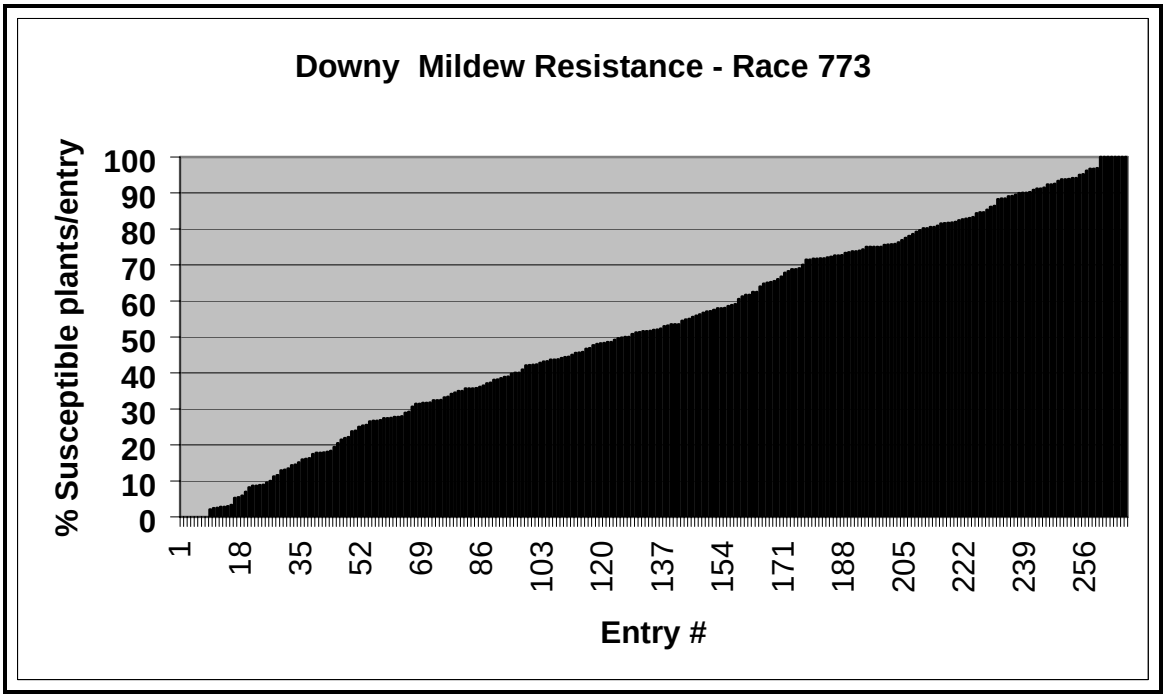

Figure 5.. Histogram showing the percentage of wild *Helianthus annuus* plants susceptible to downy mildew race 773, the most virulent race, in a test of 286 accessions.

	% Plants Susceptible to Downy Mildew Race			Origin
	300	730	773	
PI 435417	0.0	0.0	0.0	Seabrook, TX
PI 413161	0.0	2.6	0.0	Kent, TX
PI 435438	2.8	4.2	0.0	Encinal, TX
PI 435414	0.0	6.5	0.0	Paris, TX
PI 435424	5.6	9.1	0.0	Big Chocolate Bayou, TX
PI 435432	3.3	10.0	0.0	Hwy 70/77 jct, TX
PI 435378	11.5	18.1	0.0	Dodge City, KS
Ames 7075	10.3	36.1	0.0	Camps, CA

Table 1. Seven most resistant wild *Helianthus annuus* accessions showing levels of susceptibility to three races of downy mildew.

STUDY 2: Downy Mildew Resistance Screening in Other Annual *Helianthus* taxa. Resistance to an extremely virulent downy mildew race mixture (777) was found in 15 of 17 annual *Helianthus* taxa tested (Table 2). The number of accessions tested per species ranged from one (for two species) to 88. Thus, inferences about the relative frequency of DM resistance by species may be misleading when a small number of accessions were available for testing. The frequency of DM resistance by species is depicted in Figure 6, which shows that *H. argophyllus* accessions had the most resistance of any species tested. A total of 270 accessions produced sufficient seedlings for testing, and of these, 235 accessions had some percentage of seedlings resistant to race 777 DM, ranging from 2% to 100%. The species with the highest incidence of resistant plants were *H. argophyllus* and all five *H. debilis* subspecies. All of these species are normally found in beach environments or sandy habitats, primarily in Texas and Florida. The subspecies of *H. debilis* have a fairly limited geographic distribution (Figures 7), slightly more widespread than *H. agrophyllus*. For those unfamiliar with the appearance or habitat of *H. debilis*, please refer to Figure 8. *Helianthus petiolaris* also had a few highly resistant accessions, and these resistant accessions originated from Texas, New Mexico and North Dakota. A summary of the accessions of the most downy mildew resistant annual *Helianthus* speices is found in Table 3, along with the place of origin..

				Resistance	
	species	subspecies	#tested	range	average
1	anomalus		2	8 to 33%	21
2	argophyllus		13	42 to 100	83
3	debilis	debilis	10	31 to 88	65
4		cucumerfolius	6	25 to 100	77
5		silvestris	22	0 to 96	48
6		tardiflorus	4	0 to 60	21
7		vestitus	3	20 to 63	39
8	deserticola		1	0	0
9	neglectus		28	0 to 24	6
10	niveus	canescens	15	0 to 25	8
11		tephrodes	1	0	0
12	petolaris	fallax	29	0 to 63	21
13		petiolaris	88	0 to 93	28
14	porterii		8	0 to 45	14
15	praecox	hirtus	7	0 to 18	10
16		praecox	8	8 to 61	22
17		runyonii	24	0 to 68	16

Table 2. Summary of resistance to downy mildew race 777 observed in 278 accessions of 17 annual *Helianthus* taxa.

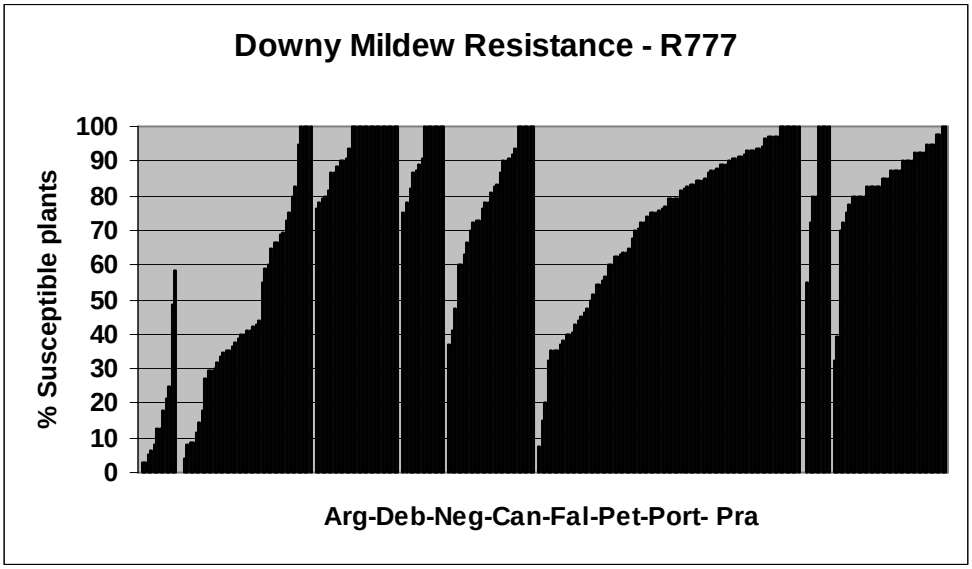

Figure 6. Histogram depicting levels of susceptibility to race 777 downy mildew observed in 270 accessions of 16 annual *Helianthus* taxa. Histograms are grouped, by species, as follows: Arg: *H. argophyllus*, Deb: all *H. debilis* ssp., Neg: *H. neglectus*, Can: *H. petiolaris ssp. canescens*, Fal: *H. petiolaris ssp. fallax*, Pet: *H. petiolaris ssp. petiolaris*, Port: *H. porterii*, Pra: all subspecies of *H. praecox*.

	Species	% Resist	Origin
PI 494580	arg	100	TX
PI 435673	deb cuc	100	GA
PI 494579	arg	97	TX
PI 494578	arg	97	TX
PI 494586	deb sil	96	TX
PI 494576	arg	95	TX
PI 494581	arg	94	TX
PI 613762	pet pet	92	ND
Ames 24595	arg	92	TX
PI 468667	deb cuc	92	SC
PI 494584	deb sil	91	TX
PI 597908	deb cuc	91	FL

Table 3. Summary of the most resistant annual *Helianthus* accessions, by species, when evaluated with downy mildew race 777. Abbreviations: arg*: H. argophyllus*, deb cuc*: H. debilis* ssp. *cucumerfolius*, deb sil*: H. debilis ssp. silvestris*, and pet pet: *H. petiolaris ssp. petiolaris*.

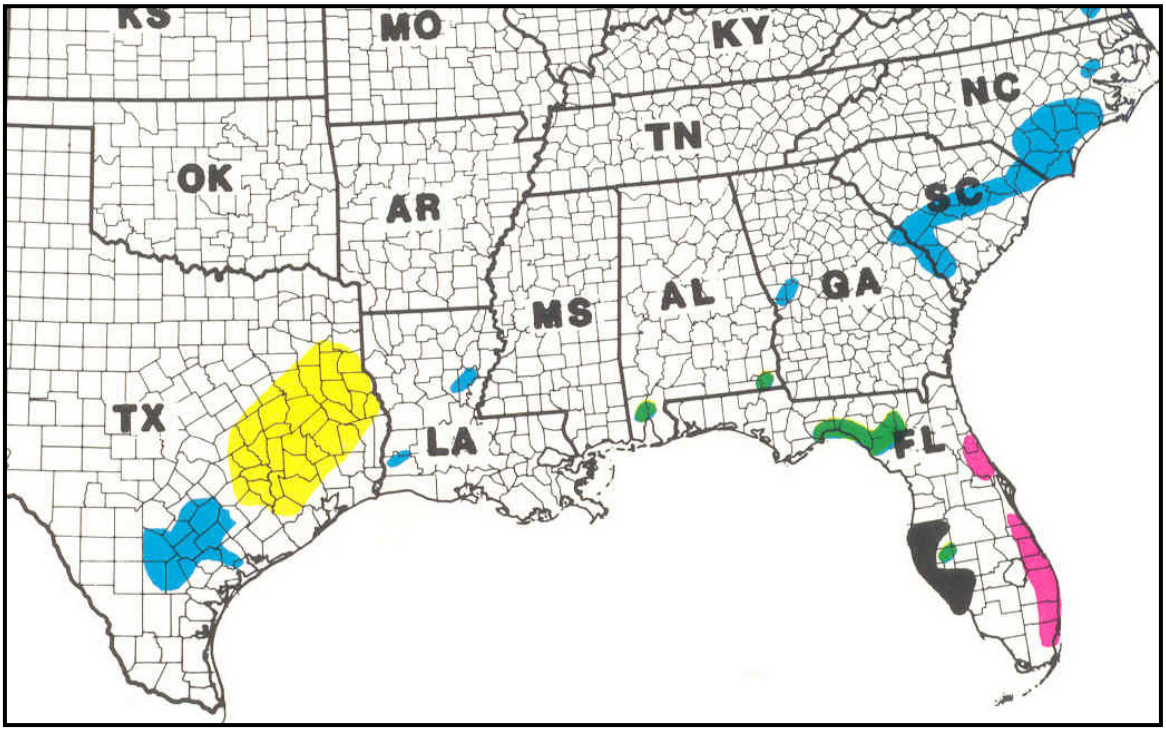

Figure 7. Distribution of *Helianthus debilis* subspecies: *cucumerfolius*: blue, *debilis*: pink, *silvestris*: yellow, *tardiflorus*: green, and *vestitus:* black. Map originally from "Sunflower species of the United States," by C.E. Rogers, T. E. Thompson, and G.J. Seiler, 1982. National Sunflower Association. .

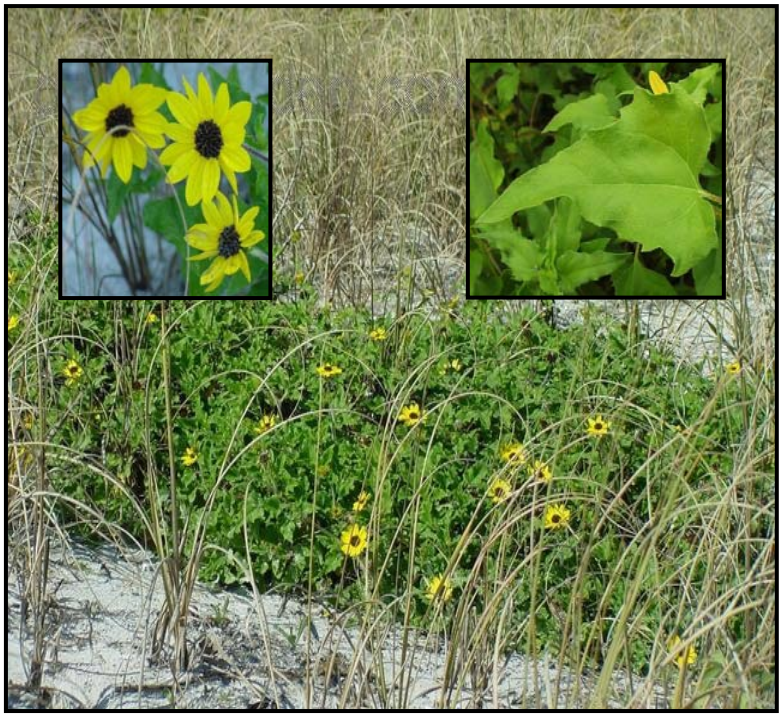

Figure 8. *Helianthus debilis* ssp. *cucumerfolius,* an annual species often with high levels of downy mildew resistance, in typical beach habitat, with closeup of flowers (left insert) and leaves (right insert)

STUDY 3: Sclerotinia Stalk Rot Resistance in Two Annual *Helianthus* Species.
This was the first greenhouse evaluation of any wild *Helianthus* species for resistance to Sclerotinia stalk rot we have conducted. This preliminary test concentrated on two recently collected species, each from unique habitats (Figures 9 and 10). A wide range in disease response was observed in the two species. Susceptibility in *H. exilis* ranged from 0 to 95% plants per accession, and from 36 to 90% in *H. porterii*. Overall, only 37% of *H. exilis* plants died from *Sclerotinia*, while 64% of the *H. porterii* plants were susceptible. Susceptible plants of *H. exilis* died faster (averaging 8 days after inoculation) compared to 13 days for *H. porterii*, but this may be related to plant phenology and not inherent resistance factors. In another study examining 12 accessions of the perennial *H. schweinitzii*, only 34% of the plants were susceptible, with individual accessions having from 13 to 88% plants susceptible. All plants of the susceptible checks (oilseed hybrids) were killed. These results are encouraging and suggest that high levels of Sclerotinia stalk rot resistance may exist in certain populations of some *Helianthus* species. Since these results are preliminary, they need to be verified both in repeated greenhouse and field trials, and in comparison with cultivated sunflower lines with known resistance.

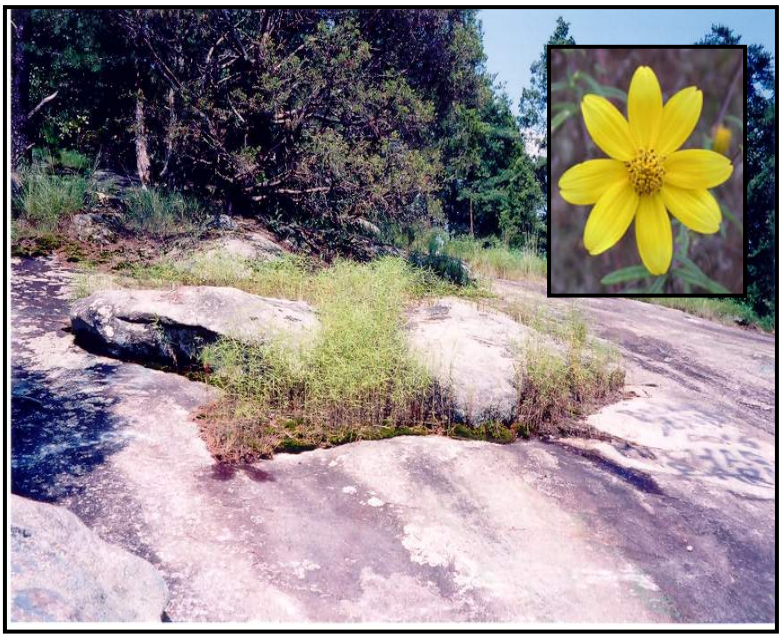

Figure 9. Typical granite rock outcrop habitat for *Helianthus porteri* (formerly *Viguiera porterii*) in the Piedmont area of northern Georgia, Alabama and adjacent South Carolina.

Figure 10. *Helianthus exilis* in a seasonal stream bed in northern California in an area with serpentine soils. Serpentine soils are abnormally high in magnesium and low in calcium.

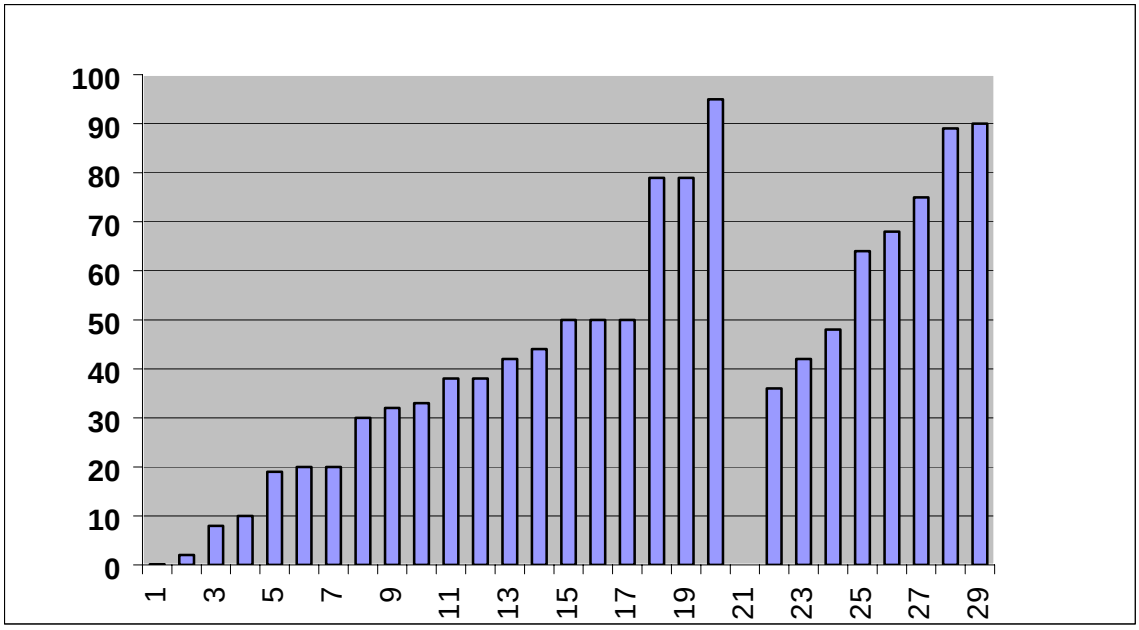

Figure 11. Histogram showing % plants susceptible to *Sclerotinia* stalk rot in greenhouse tests. *Helianthus exilis (*20 accessions) on left, *H. porterii* (8 accessions) on right

SUMMARY and CONCLUSIONS

New, more virulent downy mildew races continue to appear and increase in prevalence, rendering previously resistant sunflower hybrids vulnerable. At present, the resistance offered by the Pl_6, Pl_7 and Pl_8 genes, found in USDA releases HA 335 to RHA 340, will control all races known to exist in North America, but with the advent of race 304 in France, it is apparent that widespread use of the above genes will eventually lead to the appearance of new races which can overcome one or all of the genes. When that event occurs, the need for new downy mildew resistance genes will become critical, and this has led us to become proactive and search for new sources of downy mildew resistance. Most downy mildew resistance genes have been transferred from wild *Helianthus annuus*, with the exception of the Pl_7 gene from *H. praecox* and the Pl_8 gene from *H. argophyllus*.

With over 1,000 accessions of wild *Helianthus annuus* in the USDA-ARS NCRPIS germplasm collection from across the U.S. and Canada, there is a broad genetic base from which to search for new DM resistance genes. Looking at a cross section of 286 *H. annuus* accessions, we demonstrated that almost 10% of the accessions (26) were highly resistant to the race 773, the most virulent race in North America. These 26 annual *Helianthus* accessions all had >90% of the plants resistant, and would be immediate sources of resistance genes to transfer to cultivated sunflower. It is worthwhile noting again that the most resistant *H. annuus* most often originated from Texas. These 26 accessions are being tested further with a mixture of DM races to yield the race equivalent of 777.

In the second study we investigated the frequency of resistance to the highly virulent race 777 of downy mildew in several annual *Helianthus* taxa other than *H. annuus*. Resistance in the annual species varied, and not all taxa were represented by a high number of accessions, thus negating our ability to make generalized statements about all taxa. However, it was apparent that DM resistance was most frequent in *H. argophyllus* and several *H. debilis* subspecies, most

notably *H. debilis* ssp. *cucumerfolius*. Again, most of the highly resistant accessions originated from Texas or other warm climate locations (Table 3). In future studies examining DM resistance in the perennial *Helianthus* species, it will be interesting to observe whether DM resistance continues to be more common in species found in Texas and other southern states.

While downy mildew is controlled by single genes, and thus has been easily manipulated by plant breeders to produce totally immune hybrids, Sclerotinia head rot and Sclerotinia stalk rot resistance is polygenic, and has proven much more difficult to find and transfer resistance into hybrids. Much of the public research on sunflower worldwide has focused on Sclerotinia head rot resistance, which is the predominant phase of the disease worldwide. In the U.S., both head rot and stalk rot are of considerable importance, and since different sets of genes govern each of the two diseases, it is necessary to have two, somewhat distinct research programs searching for resistance to each type of Sclerotinia infection. Our preliminary efforts of screening wild *Helianthus* in the greenhouse for stalk rot resistance were very encouraging. We were able to evaluate young plants (4 to 6 wks old) and our inoculation method allowed us to differentiate among populations. Serpentine sunflower (*H. exilis*) appears to display a wide range of reaction to Sclerotinia stalk rot, ranging from complete susceptibility to near immunity, based on one test. We are in the process of repeating the greenhouse trials and will additionally test these populations in inoculated field trials. Stalk rot resistance was also observed in *H. porteri*, but not to the same degree as in *H. exilis*. We are also currently in the process of evaluating several hundred perennial *Helianthus* accessions. Once the resistant plants are identified, they will be crossed with a high-oleic USDA oilseed inbred, and then the F_1 plants tested for stalk rot resistance in field trials.

It continues to be apparent from these three studies that resistance to several sunflower diseases can be found in wild *Helianthus* species, both annual and perennial. Depending upon the epidemiology of the particular pathogen, resistance may be more frequent in species or populations collected from a specific geographic region of North America, as shown by the high frequency of downy mildew resistance originating from collections from Texas. There has not been enough testing of wild *Helianthus* for Sclerotinia resistance to make any definitive statements about geographic trends relative to disease resistance. With 60+ *Helianthus* taxa native to North America, the value of collecting and evaluating this untapped source of disease resistance genes is rapidly becoming apparent. Identification and utilization of these new disease resistance genes will benefit both U.S. and foreign sunflower production.