EVALUATION OF WILD SUNFLOWER SPECIES FOR DOWNY MILDEW RESISTANCE

Gerald J. Seiler and Tom J. Gulya
USDA-Agricultural Research Service, Northern Crop Science Laboratory, Fargo, North Dakota 58105

SUMMARY

Cultivated hybrid sunflower (*Helianthus annuus* L.) lacks genes for acceptable levels of resistance to [*Plasmopara halstedii* (Farl.) Berl. and de Toni], downy mildew (DM), a serious fungal disease infecting sunflower and limiting production in a majority of the sunflower producing countries. With the continued discovery of new races of DM, it is increasingly more difficult to find resistance in cultivated sunflower germplasm. The development of multiple races of DM necessitates the identification of new sources of resistance. Genetic variability for increased disease resistance in cultivated sunflower can be obtained by utilizing the numerous wild species. Twenty-five perennial and two annual species were screened for resistance to races 1 through 7 of DM using a modified leaf disk immersion technique. Pathogenicity of races was checked using standard differentials and the seedling inoculation method. Several perennial species appear to be resistant to multiple races of DM, with only one species, *H. arizonensis* R. Jackson, showing susceptibility to six races. The two annual species tested were more susceptible than the perennial species. *Helianthus debilis Nutt. ssp. debilis* was susceptible to all seven races, while *H. paradoxus* Heiser was susceptible to five races. Additional populations of perennial *Helianthus* spp. will have to be screened to determine the utility of a species. More research is needed to determine the correlation between the whole seedling and the leaf disk immersion technique, specifically for the wild perennial *Helianthus* spp.

INTRODUCTION

Sunflower diseases continue to limit sunflower production in a majority of sunflower producing regions of the world. Commercial hybrid sunflower lack genes for acceptable levels of resistance for several of the sunflower diseases. One of these

diseases is downy mildew (DM), caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni, a destructive seed-and-soil borne disease of considerable economic importance. Presently, seven races of DM have been identified, and all are found in North America (Garcia and Gulya, 1991; Gulya et al., 1991).

With the discovery of new races of DM, new genetic sources of resistance are needed. Genetic variability of cultivated sunflower for disease resistance can be increased by crossing with the numerous wild *Helianthus* species as potential sources of genes for DM resistance. The objective of the study was to evaluate wild annual and perennial sunflower species for their reaction to seven races of DM using a modified leaf disk immersion (LDI) technique.

MATERIALS AND METHODS

Twenty-five wild perennial and two wild annual species of *Helianthus* were collected from throughout the U.S. The perennial species and subspecies included: *H. angustifolius* L., *H. arizonensis* R. Jackson, *H. atrorubens* L., *H. ciliaris* DC., *H. decapetalus* L. (two populations), *H. divaricatus* L., *H. eggertii* Small, *H. giganteus* L. (three populations), *H. glaucophyllus* Smith, *H. grosseserratus* Martens, H. *hirsutus* L., *H. laciniatus* A. Gray, *H. X laetiflorus* Pers., *H. laevigatus* T.& G., *H. maximiliani* Schrad., *H. microcephalus* T.& G., *H. mollis* Lam., H. *nuttallii* T.& G. *ssp. rydbergii* (Britton) Long. (two populations), *H. occidentalis ssp. occidentalis* Riddell, *H. occidentalis Riddell ssp. plantagineus* (T. & G.) Heiser, *H. pumilus* Nutt., *H. pauciflorus (=rigidus) ssp. pauciflorus* Nutt., *H. pauciflorus* Nutt. *ssp. subrhomboideus* (Rydb.) Spring & E. Schilling (two populations), *H. salicifolius* Dietr., *H. silphioides* Nutt., *H. smithii* Heiser, and *H. X multiflorus* L. The annual species studied were *H. debilis* Nutt. ssp. *debilis*, and *H. paradoxus* Heiser. The perennial species were established in the USDA-ARS wild sunflower perennial nursery at Bushland, Texas. Rootstocks of the species were transplanted into pots and grown in the greenhouse before being evaluated. The annual species were planted at the same time as the perennial rootstocks. Seven North American races (one through seven) were used in the study (Gulya et al., 1991). The leaf disk immersion technique of Sackston and Vimard (1988) was used with modifications by Gulya (1990). Well expanded, healthy lower leaves, were used for obtaining the leaf disks. Three leaves were taken from each species, one from each plant. The leaves were rinsed in tap water, then dusted with carborundum (400-mesh) which was rubbed into the leaf surface to abrade the

epidermis, and then rinsed again. From each leaf, two 10-mm disks were cut with a sterile cork borer, one on each side of the mid-vein for a total of six disks per species. Disks were immersed in 50ml of a spore suspension (50,000 zoosporangia ml^{-1}) in a side-arm flask for 30 seconds under a vacuum of 23 mm Hg. The water-soaked leaf disks were placed in a petri dish with fresh inoculum in the dark for 3 hours at 15 to 20 C. After 3 hours, disks were removed from the inoculum, blotted dry, placed in a petri dish containing 0.7% water agar, and sealed with parafilm. The dishes were placed in a growth chamber at 15 C with a 16 hour light and 8 hour dark cycle for 7 days. After 7 days, disks were evaluated for infection by observation under a dissecting microscope at 25-40X. This procedure was repeated for each of the seven DM races. Pathogenicity of the races was checked using differentials and the whole seed immersion (WSI) technique. For interpretation of data in this study, species were rated susceptible if any disk had sporangia and resistant if all of the disks lacked sporangia.

RESULTS

Eleven perennial species were resistant to all seven races of DM tested. Another ten species were resistant to six races. The most susceptible perennial species was *H. arizonensis*, which was susceptible to all DM races, except race 5. Another susceptible perennial species was *H. X multiflorus* (hybrid of *H. annuus* and *H. decapetalus*) which was susceptible to all races, except race 3 and 7. The susceptible perennial species were most frequently susceptible to race 1 and 5 (eight species each), and race 2 (six species).

The annual species *H. debilis ssp. debilis* was susceptible to all races of DM. The other annual species tested, *H. paradoxus*, was susceptible to races 2, 3, 4, and 5. Adequate leaf material of this species was not available to test for all races.

DISCUSSION

The high degree of susceptibility of *H. arizonensis* is interesting. This species is relatively restricted (endemic) in its distribution and is habitat specific, growing in dry sandy soil in New Mexico and Arizona. The environmental conditions may

not be conducive for DM development, therefore the species may not have had to develop any level of resistance because of the absence of the pathogen. *Helianthus X multiflorus* was another perennial with a high degree of susceptibility. Its hybrid origin (*H. annuus* X *H. decapetalus*) may account for its susceptibility, especially from the annual species. The two annual species examined did not appear to have resistance. Annual *H. paradoxus* is also a species with restricted distribution in moist saline habitats, but in a desert environment. The other annual species *H. debilis ssp. debilis* grows in the coastal areas of Florida, areas which should be conducive for DM.

Race 1 of DM, commonly referred to as the European race, has only been verified from one location in Quebec, Canada in North America. Therefore, the frequency of susceptibility of wild perennial species to this race in North America may be due to lack of exposure to the pathogen. There appears to be a high level of resistance in the perennial species (24 of 32 accessions) to race 5, the most virulent DM race.

Since wild perennial and annual species of sunflower are open-pollinated, they are usually heterogeneous segregating populations. Some populations have more resistance than others. In some species where more than one population was evaluated, we observed resistance for a specific race in one population but not in the other, e.g. *H. nuttallii ssp. rydbergii* (races 1 and 5). Different reactions of the subspecies of *H. occidentalis* for race 1 were also observed. In contrast, all three populations of *H. giganteus* were resistant to all seven DM races. It is possible that additional populations will need to be examined before making a conclusive statement about the level of resistance in a species and its utility as a source of resistance.

The high frequency of resistance in perennial species is noteworthy. *Plasmopara halstedii* is reported to infect many genera of the Asteraceae Family, including both annual and perennial *Helianthus* species. Novotelnova (1966), however, has proposed taxonomic forms of the pathogen which are specialized to attack either annual or perennial *Helianthus*. It may be possible that the DM races used in this study are more specific to the annual species than the perennial species. It is also conceivable that DM isolated from a perennial *Helianthus* could be more pathogenic, but this has not been determined. The fact remains that using DM isolates increased on annual cultivated sunflower, we have observed susceptibility of wild perennial species to some races.

The definition of susceptible and resistant in the present study using the modified LDI results is quite strict. We did see varying levels of infection per leaf disk and frequency of infection. If any of the six disks of a species were infected, it was considered as susceptible.

Table 1. Wild sunflower species collected from the Central Great Plains Region of the U.S. during September, 1991.

Species	Number of populations	Collection sites(s)[a]	Typical habitat
ANNUAL			
H. annuus	82	ND,SD,NE,KS, MT,WY,CO	Disturbed roadside ditches
H. petiolaris ssp. *petiolaris*	25	ND,SD,NE,KS, MT,WY,CO	Sandy roadside ditches
PERENNIAL			
H. grosseserratus	3	NE	Moist lowlands and prairies
H. maximiliani	16	ND,SD,NE,MT	Wet to dry roadside ditches
H. nuttallii ssp. *nuttallii*	3	WY,CO	Moist roadside ditches
H. nuttallii ssp. *rydbergii*	3	ND	Moist roadside ditches Sandy soil
H. pauciflorus ssp. *subrhomboideus*	6	ND,SD,NE	Dry sandy roadside ditches
H. pumilus	1	CO	Sandy roadside ditches
H. tuberosus	1	NE	Moist roadside ditches
Total	140		

[a] ND = North Dakota, SD = South Dakota, NE = Nebraska, KS = Kansas, MT = Montana, WY = Wyoming, and CO = Colorado.

The LDI technique is a labor saving method compared to the classical WSI for perennial sunflower species. It is very difficult to obtain adequate numbers of uniform age seedlings of perennial species to test using the WSI technique. Limited information is available correlating the two methods. This will have to be done to validate the degree to which the LDI can be used in a selection program. This will be the subject of future studies.

CONCLUSIONS

Eleven perennial species were resistant to all seven races of DM. The perennial species were most susceptible to races 1 and 5, followed by race 2, using the modified LDI technique. *Helianthus arizonensis* was susceptible to six races of DM. The annual species *H. debilis ssp. debilis* was susceptible to all seven races of DM. Since all wild sunflower species are open-pollinated heterogeneous segregating populations, additional populations of wild species will have to be examined before making conclusive judgement as to the utility of a species. The leaf disk technique for screening wild perennial sunflower appears to be useful because it was able to identify both resistant and susceptible sources of DM. More studies will have to be undertaken to determine the level of correlation between WSI and LDI techniques.

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