PREVALENCE OF SUNFLOWER DOWNY MILDEW AND PATHOGEN VIRULENCE IN THE UNITED STATES NORTH CENTRAL GREAT PLAINS

Michelle A. GILLEY[1], Christopher G. MISAR[2], Thomas J. GULYA (Retired)[2], Samuel G. MARKELL[1]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND, USA;
[2]USDA-ARS Northern Crop Science Laboratory, Sunflower and Plant Biology Research Unit, Fargo, ND, USA

*michelle.gilley@ndsu.edu

ABSTRACT

Genetic resistance is one of the most important management tools of sunflower [*Helianthus annuus* L.] downy mildew caused by *Plasmopara halstedii* [(Farl.) Berl. and de Toni]. However, many resistance genes have been overcome by the pathogen and the incorporation of additional resistance genes into commercial hybrids is needed. Assessment of pathogen virulence is critical for determining what resistance genes should be incorporated into hybrids. The objectives of this study were to determine the prevalence of downy mildew and determine the virulence of *P. halstedii* isolates collected from United States (U.S.) north-central Great Plains states. In 2014 and 2015, 105 and 76 fields, respectively, were surveyed in North Dakota and South Dakota by visually assessing 40 plants at five locations for signs and symptoms of downy mildew. In 2014, 65% of those fields had downy mildew and ten fields (10%) had field-wide incidence levels higher than 5%. In 2015, 78% of fields had downy mildew and sixteen (21%) had field-wide incidence levels higher than 5%. To determine the virulence phenotypes of *P. halstedii*, 185 pathogen samples were evaluated on the international standard nine *P. halstedii* differentials and up to 13 supplemental lines were evaluated as additional differential candidates containing additional resistance genes. Virulence was observed on all nine differential lines and some candidate differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that the inclusion of additional differentials is needed.

Key words: Downy mildew, *Plasmopara halstedii*, Races, Resistance genes, Sunflower, Virulence phenotype

INTRODUCTION

Downy mildew caused by the biotrophic, Oomycete pathogen, *P. halstedii* (Farl.) Berl. and de Toni, is an economically significant seedling disease of cultivated sunflower, *Helianthus annuus* L., grown in temperate regions. Cool temperatures around 11°C and wet soil conditions between germination and emergence favor infection of sunflower radicles by *P. halstedii* zoospores (Baldini et al. 2008). If seedlings do not damp-off, cotyledons and the first true leaves become thickened, puckered and chlorotic (Gulya et al. 1997). Later, leaves show chlorosis along the veins and across the leaves while mycelia and zoosporangia appear on the underside of the leaves below the chlorotic areas. Plants that survive are severely dwarfed with shortened internodes and horizontal heads. Yield losses due to downy mildew are dependent on the number of systemically infected plants and their distribution within the field (Friskop et al. 2009).

Qualitative genetic resistance is one of the most important management tools for sunflower downy mildew; however, many previously deployed, single, dominant resistance genes (denoted *Pl*) have been overcome by the pathogen (Tourvieille de Labrouhe et al. 2008). Therefore, the incorporation of additional resistance genes into commercial hybrids as well as the use of fungicidal seed treatments has been and continues to be necessary. A field survey was conducted in order to determine the effectiveness of sunflower downy mildew control in the main production area of the U.S., the north-central Great Plains. Assessment of pathogen virulence is critical for evaluating effectiveness of resistance genes which have been incorporated into hybrids; therefore, a set of nine internationally recognized differential lines became standard in 2000 to identify virulence phenotypes or races of sunflower downy mildew (Tourvieille de Labrouhe et al. 2000). In 2012, Institut National de la Recherche Agronomique (INRA) proposed two additional sets of three differentials to update the race nomenclature bringing the total number of digits in the virulence phenotype code to five (Tourvieille de Labrouhe et al. 2012). These differentials and up to seven supplemental lines lines containing additional sources of resistance were evaluated to determine their effectiveness as additional differential candidates containing additional resistance genes.

MATERIALS AND METHODS

From June 30 to July 10, 2014 and July 8 to 24, 2015, 105 and 76 fields, respectively, were surveyed in the states of North Dakota and South Dakota. To determine field incidence, a visual inspection was made for downy mildew symptoms of 40 plants at five points in an inverted W-shaped pattern for a total of 200 plants. Prevalence was determined based on whether the disease was present or absent in the field. Pathogen isolates were collected from each field for a total of 436 isolates. An additional 125 viable isolates from North Dakota, South Dakota, Minnesota and Nebraska were collected and sent in by personnel from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), state Extension services, and seed companies.

In order to increase *P. halstedii* samples collected, susceptible sunflower seedlings were inoculated in a zoosporangia suspension prepared from symptomatic leaves for three to six hours using methods described by Gulya (1996). Inoculated seedlings were planted in a sand-perlite mixture and grown in the greenhouse for eight to ten days. Then, seedlings were placed in a glass chamber in a cool (16-18^{o}C) room and sprayed with a fine mist of water to achieve 100% relative humidity for 16 to 48 hours to induce sporulatation. The cotyledons covered with zoosporangia were harvested, desiccated, and stored in cryotubes at -80^{o}C.

After inoculum increases were completed, one isolate from each field or research plot was arbitrarily selected for virulence phenotyping. In total, 185 isolates were evaluated on the nine international standard *P. halstedii* differential lines and up to thirteen supplemental lines containing additional resistance genes. Differential seedlings were inoculated and planted using the previously described method. After 11 to 14 days, when true leaves were easily visible, sporulation was induced. Plants were allowed to air dry before susceptibility and resistance of plants was evaluated.

The following new rating system proposed by INRA for susceptible and resistant plants was used: RI = resistant, no sporulation; RII = weak sporulation on cotyledons; SI = susceptible, sporulation on cotyledons and true leaves and SII = abundant sporulation on cotyledons only (Tourvieille de Labrouhe et al. 2012). Moderate, easily visible sporulation on the cotyledons was considered to be a RII reaction. To determine race in the triplet code system, each set of three differential lines is given a numerical value. The first three lines correspond to the first digit (Table 1), the second three lines correspond to the second digit and the third three lines correspond to the third digit. If a line is resistant, it is given a value

of 0. Otherwise, the first line is given a 1, the second line a 2 and the third line a 4. The values for all three lines in each of the three sets are then added. Each digit ranges from 0 if all three lines were resistant to 7 if all three lines were susceptible. The proposed five-digit code adds two additional sets of three differential lines.

Table 1. Standard and Proposed International Differentials.

	Digit	Differential Line	Sunflower Line	Genes
Standard	1	1	Susceptible (MYC 270)	None
		2	RHA 265	Pl_1
		3	RHA 274	Pl_2/Pl_{21}
	2	4	DM-2	Pl_5
		5	PM 17	?
		6	803	?
	3	7	HA-R4	Pl_{16}
		8	HA-R5	Pl_{13}
		9	HA 335	Pl_6
Proposed	4	10	Y7Q	Pl_{6-}
		11	PSC8	Pl_2
		12	XA	Pl_4
	5	13	PSS2RM	Pl_6/Pl_{21}
		14	VAQ	Pl_5
		15	RHA 419	Pl_{Arg}

RESULTS AND DISCUSSION

In 2014, 65% of fields surveyed had sunflower downy mildew and 10% of fields had incidence levels greater than 5% (Table 2). In 2015, 78% of fields had downy mildew and 21% of fields had incidence levels higher than 5%. These fields did not appear to be concentrated in any particular region. Prevalence was high, but yield impacting incidence was low. Yield losses start to occur between 5 and 15% depending on the distribution of systemically infected plants within a field; therefore, if scattered infection occurs, incidence below 15% should result in minimal yield loss (Bradley et al. 2007). In 2015, most infected plants appeared to be scattered throughout the fields, so other plants should have compensated in this incidence range. Over the two years of the survey, six of 181 fields would be expected to have significant yield loss due to sunflower downy mildew.

Table 2. Prevalence and Incidence of Sunflower Downy Mildew for 2014 and 2015.

	2014	2015
Prevalence	65% (68/105)	78% (56/76)
Incidence		
0	65%	55%
0.5 - 4.5%	25%	24%
5 - 14.5%	9%	14%
≥ 15	1%	7%

Virulence was observed on all nine differential lines and some supplemental differential lines (Table 3). Minimal virulence was found on lines HA-R4 and HA-R5, which were released in 1984, containing *Pl16* (1%) and *Pl13* (1%) genes, respectively, (Liu et al. 2012; Mulpuri et al. 2009; Vear et al. 2008). In 1986, the USDA released six downy mildew resistant lines: *Pl6* in HA 335 and HA336 from wild *H. annuus*, *Pl7* in HA337, HA 338 and HA 339 from *H. praecox* and *Pl8* in RHA 340 from *H. argophyllus* (Miller and Gulya 1991). *Pl6* and *Pl7* were found to be similar (Miller and Gulya 1991). Between 2009 and 2013 nine races overcame the *Pl6* gene in the United States (Gulya et al. 2014). Isolates virulent on the *Pl6* gene have been between 38 and 60% since 2011 with an average of 51% (Gulya et al. 2014). Virulence on the *Pl6* gene was found on 47% of the isolates from 2014 and 2015. Seven isolates were found over the two years in North Dakota that were virulent on RHA 340, which contains the *Pl8* gene. No isolates were virulent on both the *Pl6* and the *Pl8* genes. Resistance genes in the supplemental lines evaluated include *PlArg* in RHA 419 and RHA 420 which was released in 1999 from *H. argophyllus*, *Pl17* in HA458 released in 2006 from wild *H. annuus*, an unknown gene in RHA 468 released in 2006, *Pl18* in HA DM 1 released in 2015 from *H. argophyllus* and *Pl15* in RNID a proprietary inbred line from NIDERA in Argentina (DuBle et al. 2004; Paniego et al. 2012; Qi et al. 2015, 2016; Vear et al. 2008). No virulence was found on six supplemental lines containing *PlArg*, *Pl15*, *Pl17*, *Pl18* and two other lines with unknown resistance genes.

Table 3. Results for Standard and Supplemental Sunflower Downy Mildew Differential Lines for 2014 and 2015.

Differential Line		Sunflower Lines	Genes	2014 Isolates Virulent / Isolates Screened	2015 Isolates Virulent / Isolates Screened	Total Isolates Virulent /Isolates Screened	Percent
Standard	1	Susceptible (MYC 270)	None	105/105	80/80	185/185	100%
	2	RHA 265	*Pl1*	105/105	80/80	185/185	100%
	3	RHA 274	*Pl2/Pl21*	101/105	70/80	171/185	92%
	4	DM-2	*Pl5*	83/105	56/80	139/185	75%
	5	PM 17	?	10/105	4/80	14/185	8%
	6	803	?	9/105	3/80	12/185	6%
	7	HA-R4	*Pl16*	1/105	1/80	2/185	1%
	8	HA-R5	*Pl13*	1/105	1/80	2/185	1%
	9	HA 335	*Pl6*	53/105	34/80	87/185	47%
Supplemental		RHA 340	*Pl8*	2/105	5/80	7/185	4%
		RHA 419	*PlArg*	0/105	0/80	0/185	0%
		HA 458	*Pl17*	0/61	0/80	0/141	0%
		HA DM 1	*Pl18*	0/87	0/80	0/167	0%
		RHA 468	?	0/66	0/80	0/146	0%
		TX 16R	?	0/84	0/80	0/164	0%
		RHA 428	?	15/66	0/0	15/66	23%
		RNID	*Pl15*	0/66	0/80	0/146	0%

Based on the current standard nine *P. halstedii* differentials, twelve races were found in 2014 and 2015 in isolates from North Dakota, South Dakota, Minnesota and Nebraska (Table 4). In both years, the most common downy mildew races were 714, 710 and 700,

comprising 77% of the total. Race 774 was the 4th most frequent race in 2014, while race 314 was the 4th most frequent race in 2015. Three races, 304, 707 and 717, have been identified in France, but are new to the U.S. (Virányi et al. 2015). Seven isolates that were virulent on the *Pl8* gene are currently differentiated by the addition of a "+" following the current race type since RHA 340 has not been proposed as a differential. 58% of the 26 fields with incidence greater than 5% had races that were not virulent on the *Pl6* or the *Pl8* genes.

Table 4. ABSTRACT of Sunflower Downy Mildew Races for 2014 and 2015.

Race	2014	2015	Total
304	1	0	1
314	3	10	13
700	18	18	36
700+	1	1	2
704	1	4	5
707	1	0	1
710	32	21	53
710+	1	4	5
714	37	17	54
717	0	1	1
730	0	1	1
734	1	0	1
770	0	1	1
774	9	2	11

+ = Virulent on the *Pl8* gene

A selection of the 185 isolates collected in 2014 and 2015 were virulence phenotyped using the INRA proposed differential lines to determine how races in the main sunflower production area of the U.S. would compare to the 17 races used in the proposal (Table 5) (Gascuel et al. 2015; Tourvieille de Labrouhe et al. 2012).

Table 5. ABSTRACT of Proposed Downy Mildew Races for 2014 and 2015.

Race	2014 Isolates	Proposed Race	2015 Isolates	Proposed Race
304	1/1	30430	---	---
314	0/3	---	9/10	31430
700	7/18	70060	0/18	---
700+	1/1	70060+	1/1	70060+
704	1/1	70471	2/4	70471
707	1/1	70771	---	---
710	14/33	71060	2/20	71060
710+	1/1	71060+	3/3	71060+
710+	---	---	1/1	71070+
714	6/37	71471	2/17	71471
717	---	---	1/1	71771
730	---	---	1/1	73060
734	1/1	73471	---	---
770	---	---	1/1	77062
774	1/9	77473	2/2	77473

+ = Virulent on the *Pl8* gene

Races already evaluated by INRA were the same virulence phenotypes in the U.S., but this is the first time U.S. races 700+, 710+, 734, and 770 have been evaluated with these proposed differentials. One of the 710+ isolates from 2015 conferred virulence on multiple seed batches of Y7Q which has the postulated Pl_{6-} gene, but not on HA 335 with the Pl_6 gene.

CONCLUSIONS

Virulence was observed on all nine differential lines and some supplemental differential lines. Downy mildew remains common in the north-central U.S., and pathogen virulence on all current differential lines and additional resistance genes indicate that inclusion of additional differentials is needed. Use of resistant hybrids in combination with fungicidal seed treatments and crop rotation is currently limiting field incidence based on surveyed fields.

LITERATURE

Baldini, M., Danuso, F., Turi, M., Sandra, M. and Raranciuc, S. 2008. Main factors influencing downy mildew (*Plasmopara halstedii*) infection in high-oleic sunflower hybrids in northern Italy. Crop Protection, 27(3-5): 590-599.

Bradley, C., Markell, S., and Gulya, T. 2007. Diseases of sunflower. Pages 54-77 in: Sunflower Production Guide. D. R., Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

DuBle, C.M., Hahn, V., Knapp, S.J. and Bauer, E. 2004. Pl_{Arg} from *Helianthus argophyllus* is unlinked to other known downy mildew resistance genes in sunflower. Theor. Appl. Genet. 109: 1083-1086.

Friskop, A., Markell, S. and Gulya, T. 2009. Downy Mildew of Sunflower. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1402. Fargo, ND.

Gascuel, Q., Martinez, Y., Boniface, M.-C., Vear, F., Pichon, M. and Godiard, L. 2015. The sunflower downy mildew pathogen *Plasmopara halstedii*. Molecular Plant Pathology, 16: 109–122. doi: 10.1111/mpp.12164

Gulya, T. 1996. Everything you should know about downy mildew testing but were afraid to ask. pp. 39-48. Proc. 18th Sunflower Res. Workshop. Fargo, ND. 11-12 January. Natl. Sunflower Assoc., Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/265/Gulya_WildHelianthus_studies_05.pdf

Gulya, T., Misar, C., Markell, S., Humann, R. and Harveson, B. 2014. 2013 Update on sunflower downy mildew in the U.S.: No new races. Online Publication. http://www.sunflowernsa.com/Research/searchable-database-of-forum-papers/gulya.et.al_downymildew_poster_2014.pdf

Gulya, T., Rashid, K.Y. and Masirevic, S.M. 1997. Sunflower Diseases. In: Schneiter AA (ed) Sunflower technology and production. American Society Agronomy, Madison, Wisconsin.

Liu, Z., Gulya, T.J., Seiler, G.J., Vick, B.A. and Jan, C.C. 2012. Molecular mapping of the *Pl16* downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in sunflower. Theor. Appl.Genet. 125(1): 121-131.

Miller, J.F. and Gulya, T.J. 1991. Inheritance of resistance to race 4 of downy mildew derived from interspecific crosses in sunflower. Crop Sci. 31(1): 40-43.

Mulpuri, S., Liu, Z., Feng, J., Gulya, T.J. and Jan, C.C. 2009. Inheritance and molecular mapping of a downy mildew resistance gene, *Pl13* in cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 119(5): 795-803.

Paniego, N., Bazzalo, M.E., Bulos, M., Lia, V., Fusari, C., Alvarez, D., Altieri, E., Ramos, M.L., Galella, M.T., Kaspar, M. and Heinz, R. 2012. Genomics, mapping and marker assisted selection strategies for disease resistance. Proc. 18th Int. Sunflower Conf. International Sunflower Association, Mar del Plata, Argentina, pp. 44-50.

Qi, L.L., Foley, M.E., Cai, X.W. and Gulya, T.J. 2016. Genetics and mapping of a novel downy mildew resistance gene, *Pl18*, introgressed from wild *Helianthus argophyllus* into cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet 129: 741-752.

Qi, L.L., Long, Y.M., Jan, C.C., Ma, G.J. and Gulya, T.J. 2015. *Pl17* is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 128(4): 757-767.

Tourvieille de Labrouhe, D., Gulya, T.J., Masirevic, S., Penaud, A., Rashid, K.Y. and Viranyi, F. 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). Proc. 15th Int. Sunflower Conf. International Sunflower Association, Toulouse, France, 12-15 June, 161-166.

Tourvieille de Labrouhe, D., Serre, F., Walser, P., Roche, S. and Vear, F., 2008. Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*). Euphytica, 164(2): 433-444.

Tourvieille de Labrouhe, D., Walser, P., Jolivot D., Roche, S., Serre, F., Leguillon, M., Delmotte, F., Bordat, A., Godiard, L., Vincourt, P. and Vear, F. 2012. Proposal for improvement of sunflower downy mildew race nomenclature. Proc. 18th Int. Sunflower Conf. International Sunflower Association, Mar del Plato, Argentina, March 2012, pp. 322-327.

Vear, F., Serieys, H., Petit, A., Serre, F., Boudon, J.P., Roche, S., Walser, P. and Tourvielle de Labrouhe, D., 2008. Origins of major genes for downy mildew resistance in sunflower. Proc. 17th Int. Sunflower Conf. International Sunflower Association, Córdoba, Spain, pp. 8-12.

Virányi, F., Gulya, T.J. and Tourvieille de Labrouhe, D. 2015. Recent changes in the pathogenic variablility of *Plasmopara halstedii* (sunflower downy mildew) populations from different continents. Helia. 38(63): 149-162.