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Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in Sunflowers

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Introduction

Downy mildew caused by the obligate biotrophic pathogen, *P. halstedii* (Farl.) Berl. and de Toni, is an economically significant seedling disease in cultivated sunflowers, *Helianthus annuus* L., grown in temperate regions. Yield losses due to downy mildew are dependent on cool, wet weather between germination and emergence as well as a combination of percentage of infected plants and their location in the field (Gascuel et al. 2015; Friskop et al. 2009). Qualitative genetic resistance is one of the most important management tools of sunflower; however, many single dominant resistance genes denoted *Pl* have been overcome by the pathogen and the incorporation of additional resistance genes into commercial hybrids is needed (Tourvieille de Labrouhe et al. 2008). Assessment of pathogen virulence is critical for determining what resistance genes should be incorporated into hybrids. The objectives of this study were to monitor race changes and effectiveness of resistance genes and determine prevalence and incidence of downy mildew in North Dakota and South Dakota.

Materials and Methods

From June 30 to July 10, 2014 and from July 8 to 24, 2015, 105 and 76 fields, respectively, were surveyed in North Dakota and South Dakota. To determine field incidence, a visual inspection for downy mildew symptoms of 40 plants in 2-row pairs at five points along a W for a total of 200 plants was made. Prevalence data was based on whether the disease was present or absent in the field overall.

For most fields, up to three pathogen isolates were collected for a total of 436 isolates. USDA-ARS, extension and seed company personnel sent in an additional 125 viable samples from North Dakota, South Dakota, Minnesota and Nebraska. Susceptible sunflower seedlings were inoculated with a spore suspension prepared with symptomatic leaves to increase *P. halstedii* inoculum using similar methods to those described by Gulya in 1996. Seedlings were grown in the greenhouse for eight to ten days. Flats were placed in cool room with high relative humidity so *P. halstedii* would sporulate, then cotyledons were desiccated and frozen at -80 in cryotubes for long-term storage. One cryotube was selected from the freezer for each field or research plot and the isolate was increased on a susceptible hybrid for virulence phenotyping as well as checking for susceptibility to other resistance genes. 185 pathogen samples were evaluated on the international standard nine *P. halstedii* differentials and up to ten supplemental lines were evaluated as additional differential candidates containing additional resistance genes. Inoculated differential seedlings were placed in rows, covered with sand and watered daily. After 11 to 14 days, when true leaves were

visible, flats were misted then placed for 16 to 48 hours at 100% relative humidity at 16-18°C to cause sporulation. Flats were left on a counter to let plants dry completely before rating susceptibility and resistance. To determine virulence phenotype 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, 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 the set are then added. Each digit ranges from 0 if all three lines were resistant to 7 if all three lines were susceptible.

Results

Virulence was observed on all nine differential lines and some supplemental differential lines (Table 1). Minimal virulence was found on lines containing Pl_{16} (1%) and Pl_{13} (1%) genes. Virulence on the Pl_6 gene is holding at 47% of the isolates. Isolates virulent on the Pl_6 gene have been between 38 and 60% since 2011 with an average of 51%. Seven isolates were found over the two years in four different counties in North Dakota that were virulent on RHA 340, which contains the Pl_8 gene. No isolates were virulent on both the Pl_6 and the Pl_8 genes. All these isolates were found in fields with zero incidence except one field where it was actually a symptomatic volunteer sunflower in a soybean field. No virulence was found on six supplemental lines containing Pl_{Arg}, Pl_{15}, Pl_{17}, Pl_{18} and two other lines with unknown resistance genes.

Table 1. Results for Standard and Supplemental Lines.

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	Pl_1	105/105	80/80	185/185	100%
	3	RHA 274	Pl_2/Pl_{21}	101/105	70/80	171/185	92%
	4	DM-2	Pl_5	83/105	56/80	139/185	75%
	5	PM 17	?	10/105	5/80	15/185	8%
	6	803	?	9/105	3/80	12/185	6%
	7	HA-R4	Pl_{16}	1/105	1/80	2/185	1%
	8	HA-R5	Pl_{13}	1/105	1/80	2/185	1%
	9	HA 335	Pl_6	53/105	34/80	87/185	47%
Supplemental		RHA 340	Pl_8	2/105	5/80	7/185	4%
		RHA 419	Pl_{Arg}	0/105	0/80	0/185	0%
		HA 458	Pl_{17}	0/61	0/80	0/141	0%
		HA DM 1	Pl_{18}	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	Pl_{15}	0/66	0/80	0/146	0%

*Seed purity being evaluated

Eleven races were found in 2014 and 2015 in samples from North Dakota, South Dakota, Minnesota and Nebraska (Table 2). The current main downy mildew races are 714, 710 and 700. Three of these races, 304, 707 and 717, are new to the U.S. and only one of each was found.

Table 2. Summary of Races.

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

In 2014, 65% of the fields surveyed had downy mildew and ten fields (10%) had field-wide incidence levels higher than 5% (Table 3). In 2015, 78% of fields had downy mildew and sixteen (21%) of those had field-wide incidence levels higher than 5%. These fields did not appear to be concentrated in any one region. Prevalence was high, but yield impacting incidence was low. Yield losses start to occur somewhere between 5 and 15% depending on the disease pattern; therefore, with a scattered infection, incidence below 15% should result in minimal yield loss (Berglund 2007). In 2015, the infected plants seemed to be throughout the fields, so other plants should have been able to compensate in this incidence range. 58% of the 26 fields with incidence greater than 5% had races that were not virulent on the Pl_6 or the Pl_8 genes.

Table 3. Prevalence and Incidence of Downy Mildew

	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%

Summary

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 the inclusion of additional differentials is needed. Use of resistant hybrids in combination with fungicide seed treatments and crop rotation is currently limiting field incidence based on surveyed fields.

References

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