Races of *Plasmopara halstedii* on sunflower in separate agrocenosises of Adigeya Republic, Krasnodar and Rostov regions in Russia

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ABSTRACT

During the past four years, the occurrence of *Plasmopara halstedii* and the regional distribution of its pathotypes in some districts of Northern Caucasus and Rostov region were studied. More than 1000 isolates of the pathogen were collected in separate agrocenoses of Adigeya Republic, Krasnodar and Rostov regions in 2004-2007 period. A large-scale identification of these isolates was undertaken. Pathotype characterization, based on sunflower differential lines and evaluated according to a triplet code system, indicated the existence of at least seven pathotypes. Among them, race 330 was predominant. Races 710 and 730 dominated in some fields of Adigeya Republic and Krasnodar region. Races 100, 300, and 310 were only sporadically present. Race 700 was discovered in some fields of Krasnodar region, representing up to 11% of all isolates from these places. At present only races 330, 710 and 730 are economically important in the studied regions of Northern Caucasus.

Key words: downy mildew – *Plasmopara halstedii* – pathotypes ratio – races – sunflower.

INTRODUCTION

Downy mildew of sunflower caused by the pathogenic fungus *Plasmopara halstedii* (Farl.) Berl. et de Tony is a worldwide major disease of this crop. In a recent survey of pathogen race spreading, it was shown that more than 30 pathotypes of this Oomycete on sunflower were discovered in the World (Gulya, 2007). The quantity and composition of pathogen races vary in different countries and are the objective of study by leading phytopathologists (Masirevic, 1998; Molinero-Ruiz et al., 1998; Kormany and Viranyi, 1997; Penaud, 1998; Tourvieille de Labrouhe et al., 2000b; Shindrova, 2000, 2005; Rozynek and Spring, 2000; Shirshikar, 2005). Sunflower is the main oil crop in Russia. Adigeya Republic, Krasnodar and Rostov regions are the territories of intensive sunflower cultivation in Russia. Downy Mildew is one of the most potentially important sunflower diseases here, but for a long period of time a structure of *P. halstedii* population on sunflower in all of Northern Caucasus was a white spot on the world map of pathogen races distribution. This disease was first observed in Russia in the mid 1950s (Novotelnova, 1966). Successful development in this country of a hybrid *H. tuberosus* x *H. annuus* resistant to the disease and breeding based on its new open-pollinated varieties permitted to control the pathogen during three decades. The first information about the appearance of a new virulent pathotype of pathogen in Krasnodar region goes back to the beginning of the 1980s (Tihonov and, Zaichuk, 1981). At that time, the sunflower differential line HA-274 was resistant, but at that time both this differential line as well as the resistant varieties began to be infected. The investigations of Antonova et al. (2000) revealed the presence of races 100, 310 and 330 in Krasnodar region. The favourable weather conditions for disease appearance in 2004-2007 permitted to collect an ample collection of pathogen isolates (about 1000) in different districts of Northern Caucasus, which was preserved at a temperature of -80^{o}C, The aim of this study was to identify these isolates and to determine the race ratio in separate agrocenosises by the use of an international method proposed by a group of scientists (Gulya et al., 1998; Tourvieille de Labrouhe et al., 2000a).

MATERIALS AND METHODS

In order to determine the race variability of sunflower downy mildew in the Northern Caucasus and the ratio of the individual races in separate agrocenosises, expeditions for collecting isolates of *Plasmopara halstedii* from infected plants of different hybrids and varieties were organized in the period 2004-2007. About 1000 isolates of the Oomycete were collected from 14 regions of the Northern Caucasus (Adigeya republic, Krasnodar and Rostov areas) (Table 1, Fig. 1). Leaves from systemically infected plants in the field were harvested and kept in darkness at 6^{o}C in 100% humidity for 12 hour for induction of fungus sporulation. The leaves with sporulation were kept in polyethylene bags at -80^{o}C. At this temperature, zoosporangia do not lose their viability for some years. For differentiation of pathogen races according to the new nomenclature system, nine sunflower differential lines were used: set 1 – HA 304 (D-1), Rha-265 (D-2), Rha-274 (D-3); set 2: PM-13(D-4), PM-17 (D-5), 803-1(D-6); set 3: HAR-4 (D-7), HAR-5 (D-8), HA-335 (D-9). Races were determined on the basis of the response of the lines from each group (sporulation on the first true leaves) (Tourvieille de Labrouhe et al., 2000a). Seeds of differentials were placed for germination in rolled up filter paper at 25°C. At the radicle length of 1.0-2.0 cm the seedlings were laid in rows in growth plates with wet sterilized sand covered by filter paper (10 seedlings of each line in one plate). The roots of seedlings were covered by strips of filter paper and wet cotton wool. A suspension of zoospores was prepared from frozen spores (the concentration was 10^6 spores/ml) at 16°C; 150 ml of suspension was added to each growth plate and they were kept for 3-5 hours at the same temperature. Plants were grown 7-9 days at 25°C in the daytime and 18°C at night (16 h photoperiod). The growth plates were placed in a wet chamber at 16°C for 12-24 hours for sporulation. Plants with sporulation on true leaves were classified as susceptible. If any of the differential lines displayed partial infection, these lines were re-inoculated a second time, using spores from the universal susceptible or the line in question.

RESULTS

During the period 2004-2007, the climate conditions were favorable for downy mildew on sunflower in more regions of Northern Caucasus. Therefore, a numerous collection of *P. halstedii* isolates (more than 1000) was collected from infected sunflower plants of different varieties, hybrids and lines in 14 areas of Northern Caucasus (Fig. 1).

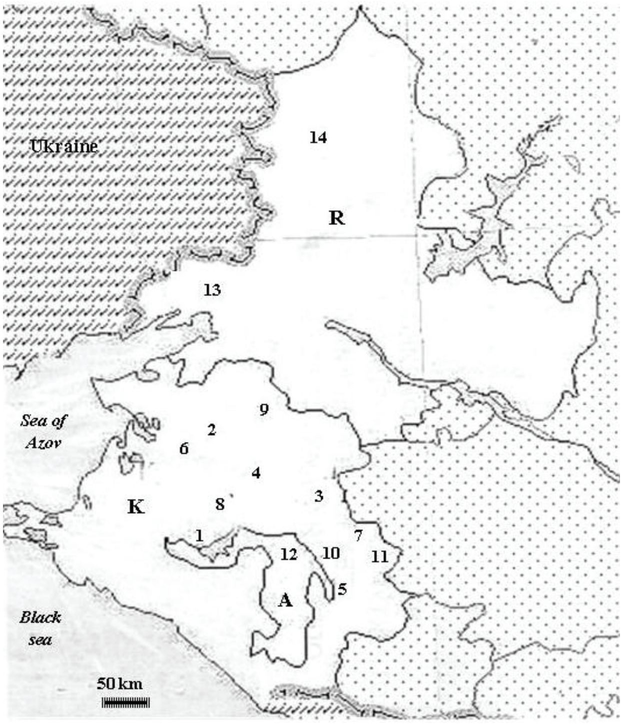

Fig. 1. Location of districts of gathering *Plasmopara halstedii* isolates from infected sunflower plants: ***K****- Krasnodar region: 1- Krasnodarskiy, 2 - Leningradskiy, 3- Caucazskiy, 4 - Viselkovskiy, 5 - Labinskiy, 6- Kanevskoy, 7 - Novokubanskiy, 8 - Korenovskiy, 9 - Krilovskoy, 10 - Kurganinskiy , 11 - Uspenskiy ;* ***A*** *- Adigeya Republic: 12- Shovgenovskiy;* ***R*** *- Rostov region: 13 -Matveev-Kurganskiy., 14 - Millerovskiy.*

Our identification of collected isolates in accordance with new nomenclature system, proposed by Tourvieille de Labrouhe et al. (2000a), was the first circumstantial investigation of race composition of *P. halstedii* population on sunflower in this part of the world. It revealed 7 races (Table 1). Pathotypes 100, 300, and 310 were only sporadically present in some areas of the above regions. Race 330 was revealed in all investigated fields everywhere (Table 1). Races 710 and 730 were discovered in 9 and 7 areas, respectively. Race 700 was revealed in Adigeya Republic and in 3 areas of Krasnodar region. All seven races were identified in fields of VNIIMK.

Table 1. *Plasmopara halstedii* races infecting sunflower in some regions of Northern Caucasus during 2004-2007.

District	Year of collection of isolates	Host of pathogen	Races						
			100	300	310	330	700	710	730
		Adigeya Republic							
Schovgenovskiy	2004	Master*	-	-	-	+	+	+	+
		Rostov region							
Matveev Kurgan	2004	R- 453 (Rodnik)*	-	-	+	+	-	-	-
Millerovskiy	2004	Signal**, Donskoy krupnoplodniy*	-	-	-	+	-	+	+
		Krasnodar region							
Leningradskiy	2004	R- 453 (Rodnik)*	-	-	+	+	-	-	-
Caucazskiy	2004	Master*	-	-	-	+	-	-	-
Viselkovskiy	2004 2005	PR64A83,** R- 453 (Rodnik)*, Signal**	-	-	+	+	+	+	+
Labinskiy	2005	R- 453 (Rodnik)*	-	+	-	+	+	+	+
Kanevskoy	2005	Master*, Flagman*, R- 453 (Rodnik)*, Signal**	+	-	-	+	-	+	+
Novokubanskiy	2005	VK 276 B***	-	-	-	+	-	-	-
Korenovskiy	2005	VK-653***	-	+	-	+	-	-	-
Krilovskoy	2006	Konditerskiy (SPK)*, Donskoy krupnoplodniy*	-	-	-	+	+	+	+
Krasnodarskiy	2004-2007	Different varieties, hybrids and lines on the fields of VNIIMK	+	+	+	+	+	+	+
Kurganinskiy	2007	Konditerskiy (SPK)*	-	-	-	+	-	+	-
Uspenskiy	2007	R- 453 (Rodnik)*	-	-	-	+	-	+	-

* open pollinated variety; **hybrid; *** inbred line.

The analysis of the race ratio was carried out for isolates of 24 separate agrocenoses. In Schovgenovskiy district of Adigeya Republic from 23 isolates of fungus, collected on one field from infected plants of open pollinated variety Master, 69.6 % belonged to race 710 and 13.0% were classified as race 330. Races 700 and 730 represented 8.7% each (Table 2). In Millerovskiy district of Rostov region (18 isolates) the highest number corresponded to races 330 (77.7%), 730 (16.7%) and 710 (5,6%). In Matveev Kurgan district, race 330 represented 80% and race 310 was 20%, but the number of isolates was small (6 isolates).

Table 2. The ratio (%) of *Plasmopara halstedii* races of infected sunflower in separate agrocenoses of Adigeya Republic and Rostov region

Location of the field	Year of collection of isolates	Host of pathogen	N. of isolates	Races				
				310	330	700	710	730
Schovgenovskiy district of Adigeya Republic	2004	Master*	23	0	13.0	8.7	69.6	8.7
Millerovskiy district of Rostov region	2004	Donskoy krupnoplodniy*	18	0	77.7	0	5.6	16.7
Matveev-Kurganskiy district of Rostov region	2004	R- 453 (Rodnik)*	6	20	80	0	0	0

*open pollinated variety

Table 3. The ratio (%) of *Plasmopara halstedii* races of infected sunflower in separate agrocenoses in different districts of Krasnodar region

N. of the field	Year of collection of isolates	Host of pathogen	N. of isolates	Races						
				100	300	310	330	700	710	730
			Viselkovskiy district							
1	2004	PR64A83**	93	0	0	1.2	23.4	11.2	60.5	3.7
2	2005	R- 453 (Rodnik)*	79	0	0	0	12.7	0	29.1	58.2
3[1]	2005	Signal**	6	0	0	0	50	0	50	0
			Kanevskoy district							
4	2005	Flagman*	60	0	0	1.7	91.7	0	3.3	3.3
5	2005	R- 453 (Rodnik)*	63	0	0	1.6	98.4	0	0	0
6	2005	R- 453 (Rodnik)*	12	0	0	0	100	0	0	0
7	2005	Master*	14	7.1	0	0	92.9	0	0	0
8[1]	2005	Signal**	12	0	0	0	100	0	0	0
			Krilovskoy district							
9	2006	Konditerskiy (SPK)*	82	0	0	0	90.2	1.2	3.7	4.9
10	2006	Donskoy krupnoplodniy*	15	0	0	0	53.3	0	26.7	20
11[1]	2006	Konditerskiy (SPK)*	11	0	0	0	100	0	0	0
			Kurganinskiy district							
12	2007	Konditerskiy (SPK)*	43	0	0	0	97.7	0	2.3	0
			Krasnodarskiy district (fields of VNIIMK)							
13	2005	VK-678*** (first planting date)	28	0	0	3.6	89.3	0	7.1	0
14	2005	VK -678*** (second planting date)	45	0	0	2.2	55.6	0	11.1	31.1
15	2005	Different genotypes	30	3.3	3.3	6.7	73.4	3.3	3.3	6.7
16	2005	R- 453 (Rodnik)*	12	0	0	8.3	66.7	0	0	25
17	2005	Different genotypes	10	0	0	0	40	0	30	20
18	2005	Different genotypes	29	0	3.5	10.3	58.6	3.5	17.2	6.9
19	2006	Different genotypes	7	0	0	0	100	0	0	0
20	2006	Different genotypes	15	0	0	0	100	0	0	0
21	2006	Volunteer plants	14	0	0	0	100	0	0	0

[1]Treated fields with metalaxyl-protected seeds; *open pollinated variety; **hybrid; *** inbred line.

Race 330 dominated in 18 out of the 21 studied agrocenoses in Krasnodar region and constituted 40-100% (Table 3). In Viselkovskiy district, two adjacent fields were analyzed. In the first field 93 isolates of the pathogen were collected from infected plants of hybrid PR64A83 in 2004. In the second field (adjacent to the first), 79 isolates were collected from open pollinated variety R- 453 (Rodnik) in 2005. In the first field, race 710 dominated (60.5%), whereas in the second dominated race 730 (58.2%) (Table 3).

Race 730 in the first field only accounted for 3.7%, but in the second field it represented 58.2%. Race 330 was 23.4 % in the first field and 12.7% in the second. Races 310 and 700 were only identified in the first field (1.2% and 11.2%, respectively). In the third field, treated with metalaxyl-protected seeds of hybrid Signal, only 6 isolates were collected and identified, with races 330 and 710 representing 50% each.

Another race composition was found on five fields of Kanevskoy district. Race 330 here was up to 100%. Races 710 and 730 were discovered only in one field, each of them made up to 3.3%. Race 100 made up to 7.1% on another field and race 310 represented 1.6 and 1.7 % in two fields. Races 300 and 700 were not found in these five fields (Table 3).

In Kurganinskiy district, the percentage of race 330 was 97.7% and race 710 constituted 2.3% (Table 3). In all the three studied fields in Krilovskoy district, race 330 also dominated (53.3-100%). From the 82 isolates collected from infected plants of variety Konditerskiy (SPK) races 700, 710 and 730 were also identified, representing only 1.2, 3.7, and 4.9%, respectively. The percentage of race 710 was 26.7% and of race 730 20% in 15 collected isolates from infected sunflower of variety Donskoy krupnoplodniy (Table 3).

All isolates from Krasnodarskiy district were collected from infected plants of different genotypes of sunflower (Russian and foreign open pollinated varieties, hybrids, inbred lines) in VNIIMK`s fields. In all 8 presented agrocenoses, race 330 predominated over the other races. The line VK-678 was planted in one field at two different planting dates. For both dates, apart from race 330, also were present races 310 and 710. Race 730 was not observed among the samples on the first date of planting but it was identified in the samples on the second date of planting (31.1%) . In one of the fields, all the 7 races were observed: races 100, 300, 700 and 710, represented each 3.3% of the samples from this field, races 310 and 730 made up to 6.7% each. In one of the remaining fields, 6 races were discovered; races 300 and 700 constituted 3.5 % each, race 310 was 10.3%, race 710 was 17.2%, and race 730 was 6,9% (Table 3).

DISCUSSION

According to this observation, seven races *of Plasmopara halstedii* were identified in regions of Northern Caucasus during the last four years. The distribution area and the ratio of pathotypes in separate agrocenoses were different. We assumed that at present races 100, 300, and 310 are disappearing here because of the following reasons: firstly, they have been discovered only sporadically; secondly, all their isolates form an extremely poor sporulation even on susceptible differential lines. In addition, races 100 and 300 are the oldest races, at least in Krasnodar territories. Prolonged sunflower breeding for resistance to these pathotypes over the span of some decades must result in their maximal ousting from the populations. At present, the main race which is widely distributed everywhere in the studied territories is 330. This race dominates up to 100% in 18 out of 21 studied agrocenoses in Krasnodar region and in the two studied agrocenosises in Rostov region. But in separate agrocenoses of Adigeya Republic and Krasnodar region, races 710 and 730 are dominant. Race 700 was found in 5 areas and it represented up to 11.2%. Its condition in pathogen population here is not clear now. Maybe it will spread more widely in the future. At present, only races 330, 710 and 730 are economically important in these territories of Northern Caucasus. Strictly speaking, the quantity of races of pathogen depends on the diversity of cultivated sunflower genotypes. The rapid change over the last decades of sunflower crop variety structures in the studied territories, together with a wide distribution of foreign hybrids everywhere in Russia, will change the race structure of *P. halstedii* populations in this country. Data from our investigation showed the necessity to concentrate efforts on the development of native sunflower varieties and hybrids resistant to races 330, 710 and 730. In addition, our investigation will serve as a starting point for controlling the population structure of *Plasmopara halstedii* in Northern Caucasus territories.

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