THE VIRULENCE OF PLASMOPARA HALSTEDII IN THE SOUTHERN REGIONS OF RUSSIAN FEDERATION

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ABSTRACT

The population of oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni (sunflower downy mildew causal agent) has been monitored in Krasnodar Krai, Rostov region and Republic of Adygea more than 15 years. Prior to the beginning of the 2000s there were races 100, 300, 310 and 330 in the region. In the period from 2004 to 2007 races 100, 300, 310 and 700 met sporadically. The race 330 was the most common; in a number of agrocoenoses it was 100 % of samples. In some fields races 710 and 730 prevailed. In 2008-2011 only races 330, 710 and 730 were found; the race 330 still prevailed and was also found on *Ambrosia artemisiifolia* L. Since 2012 in the majority of fields races 710 and 730 prevailed, and the race 330 wasn't allocated in many of them; for the first time in Russia pathotype 334, that able to overcome Pl_6, was found in Krasnodar Krai. In the period of 2013-2015 increased distribution of the race 334 in Krasnodar Krai and Republic of Adygea was observed. At the same time, in 2014 in one field in the Rostov region only races 310 and 330 (prevailed) were identified. The virulence of the pathogen population is closely connected with the cultivated assortment of sunflower. Further spread and accumulation of P. halstedii race 334 and the emergence of new pathogen pathotypes in the said regions are predicted.

Key words: downy mildew, *Plasmopara halstedii*, races, sunflower, *Helianthus annuus, Ambrosia artemisiifolia*

INTRODUCTION

One of the most spread and harmful diseases of sunflower (*Helianthus annuus L.*) in Russia is downy mildew, caused by oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni. The population of the parasite has been studied in the south regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea) more than 15 years.

Initially, in the territory of the former USSR there was only race 100. However, in the early 1980s all resistant sunflower varieties of domestic selection became affected in the Krasnodar region (Tikhonov, Zaychuk, 1984). Prior to the beginning of the 2000s there were identified races 100, 300, 310 and 330 in the region (Antonova, 2003). It should be noted that over the previous decade foreign sowing material of sunflower was freely delivered in the country and decline in terms of return of the culture in field has become the norm.

In the period from 2004 to 2007 seven races of *P. halstedii* were found in the said regions. The most common was the race 330; in several agrocoenoses it was 100 % of samples. Races 710 and 730 prevailed in some fields. Races 100, 300, 310 and 700 met sporadically. Status of the pathogen population in the region in those years was described in detail previously (Antonova et al., 2008).

The aim of our study was to monitor the racial structure of *P. halstedii* population in the southern regions of Russian Federation (Krasnodar Krai, Rostov region and Republic of Adygea).

MATERIALS AND METHODS

The leaves from the infected by downy mildew sunflower plants were collected from the fields in Adigeya republic, Krasnodar and Rostov areas in 2009-2015 (table 1).

Table 1. The total numbers of identified *P. halstedii* isolates and fields, where isolates of *P. halstedii* were collected, in different years

Total number	Years		
	2009-2011	2012-2013	2014-2015
identified *P. halstedii* isolates	196	474	480
surveyed fields	11	16	19

For identification of pathogen races, according to the nomenclature system (Tourvieille et al., 2000), nine *P. halstedii* differential lines of *H. annuus* were used: set 1 – VNIIMK 8883 (D-1), RHA-265 (D-2), RHA-274 (D-3); set 2: DM-2 (D-4), PM-17 (D-5), 803-1(D-6); set 3: HA-R4 (D-7), HA-R5 (D-8), HA-335 (D-9). The line HA-304 (D1), that was not stable in reaction of resistance or susceptibility, has been changed on the universally susceptible variety VNIIMK 8883.

Pre-germinated seeds of differentials with radicle length 1,0-2,0 cm were placed in plastic growth trays with sterilized sand, covered by filter paper. The radicles of seedlings were covered by wet cotton wool. 150 ml of zoosporangial suspension of isolates (concentration about 10^6 zoosporangia/ml) were added in growth trays (one *P. halstedii* isolate per tray) and incubated 16-20 hours at the temperature 16 ºC. Inoculated sunflower plants were grown at the temperature 25±2 ºC (16 h photoperiod) and after 7-9 days were placed in darkness at 16 ºC in 100 % humidity overnight for induction of *P. halstedii* sporulation. Plants with sporulation on leaves or with abundant sporulation on cotyledons only were classified as susceptible.

RESULTS

Until 2007 in the southern regions of Russia (Krasnodar Krai, Rostov region and Republic of Adygea) seven races of *P. halstedii* were found. Among them during 2004-2007 races 100, 300, 310 and 700 constituted together about 2,5 %, and the most common were races 330, 710 and 730 (about 65, 13,5 and 19 % respectively).

Since then, there have been significant changes in the structure of the pathogen population. They are shown in table 2, which presents the prevalence and frequency of occurrence of races in the region in different years.

Table 2. The distribution of *Plasmopara halstedii* races on sunflower in southern regions of Russia during 2004-2015*

Races	Years											
	2004-2007			2009-2011			2012-2013			2014-2015		
	F	I	Ifi	F	I	Ifi	F	I	Ifi	F	I	Ifi
100	0.05	0.2	3.3-7.1**	0	0	0	0	0	0	0	0	0
300	0.07	0.7	1.2-3.5	0	0	0	0	0	0	0	0	0
310	1.6	0.9	1.2-10.3	0	0	0	0	0	0	5.3	1.8	17.2
330	100	65.1	12.7-100	100	46.5	25.0-92.0	75.0	18.5	7.7-25.6	31.6	26.2	10-91.4
700	1.3	0.7	1.2-12.7	0	0	0	0	0	0	0	0	0
710	70	13.6	2.3-69.6	100	25	3.3-57.1	100	35.7	26.6-90.0	73.7	24.0	5.0-54.0
730	56	18.8	3.3-58.2	100	28.5	6.7-52.1	93.8	44.5	16.7-70.2	73.7	30.5	10.0-64.0
334	0	0	0	0	0	0	12.5	1.3	0.4; 2.2	47.4	17.5	20.0-100

* F - the frequency of the race occurrence in the fields, %;

I - race proportion in the total number of identified *P. halstedii* isolates, %;

Ifi - minimum and maximum percents (%) of the race in positive samples, %

**- the samples of *P. halstedii* isolates were small

Race 100, 300 and 700 were not found after 2007. However oospores of *P. halstedii* are capable of being viable in the soil up to 10 years (Viranyi and Spring, 2011). Therefore it is not excluded that these races still are present in the region as another one of the old races - 310, which was found in one field in 2014 and amounted to 17 % of the sample (table 2). The period of existence of these races in the pathogen population prolongs by cultivation of susceptible sunflower in separate fields.

Till 2011 race 330 was found in each of surveyed fields and dominated in the south of Russia. But from 2012 its part in the pathogen population has considerably decreased: it became less than 20 % in 2012-2013 and less than 30 % in 2014-2015. In 2012-2013, it was present in 75 % of samples, in 2014-2015 – only in 32 %. At the same time, race 330 has been found on plants of common ragweed (*Ambrosia artemisifolia* L.) in Krasnodar Krai in different years (2011, 2013 and 2015). All isolates collected by us from ambrosia belonged only to this race. Analyze of SNP DNK locuses proved identity of this isolates and isolates of the race 330 from sunflower (Iwebor et al., 2012). Thus the race 330 can persist in local population of *P. halstedii* on ambrosia. Even the widespread cultivation of sunflower, resistant to this race, will not lead to its complete disappearance, as in Russia *A. artemisiifolia* is ubiquitous in areas of sunflower cultivation.

Races 710 and 730 were found only in 70 and 56 % (respectively) of the surveyed fields in 2004-2007 and they were found almost in every field in 2009-2013. Since 2012 these two races (individually or together) prevailed over race 330 in pathogen population both in general and in the majority of separate agrocoenosises.

In 2012 one isolate of race 334 was discovered in Krasnodar Krai. In Russia it was the first time of detection of the pathotype that able to overcome the resistance gene Pl_6 of sunflower. In 2013 the race 334 has been found again in one field. In 2014-2015 increased distribution of this race was observed in Krasnodar Krai and in Republic of Adygea (tables 3 and 4). It was present in almost half of surveyed fields and reached 17,5 % of the total number of identified pathogen isolates. Race 334 ranged from 20 to 100 % in the samples from different field (table 2).

All changes which have happened in racial structure of *P. halstedii* population were closely connected with cultivated assortment of sunflower that was clearly demonstrated in the tables 3 and 4.

Table 3. Races of *P. halstedii*, found in the sunflower fields in the Republic of Adygea and Rostov region in 2011-2015

Location of the field	Year	Foreign hybrids of sunflower in the field*	The number of isolates					
			total	race				
				310	330	710	730	**334**
Rostov region	2011	-	19	0	6	6	7	0
	2014	-	55	0	40	7	8	0
		-	64	11	53	0	0	0
Republic of Adygea	2012	-	28	0	7	11	10	0
	2014	+	10	0	0	5	2	**3**
	2015	+	16	0	0	3	3	**10**

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): ‘+’ – yes, ‘-’ – no

In one of the sunflower fields in the Rostov region (2014), race 330 dominated and race 310 has been found. Races 710 and 730 have not been revealed there (table 3). From the history of the field it is known that only domestic sunflower varieties were cultivated there. In the other two fields of this region and in one of the fields in Adygea (2012) also domestic varieties and hybrids were grown only. There were identified races 330, 710 and 730. Race 334 was found in the Republic of Adygea in two fields, in which during several last rotations of sunflower foreign hybrids were cultivated.

The similar situation was observed in fields of Krasnodar Krai (table 4). In the fields, where only domestic varieties and hybrids have been cultivated (at least 5 last years before the year of sampling), races 330, 710 and 730 were isolated, but not the race 334.

Race 334 was revealed in the fields where in any of last 5 years (before the year of sampling) foreign hybrids of sunflower have been cultivated. It was such in the fields in Korenovsky and Tbilissky districts (Krasnodar Krai), where over the last 5 years foreign hybrids were sowed twice. In several samples race 334 made 100 %: these *P. halstedii* isolates were collected from the foreign sunflower hybrids with Pl_6 – the gene of resistance to all parasite landraces except 334.

Table 4. Races of *P. halstedii*, found in the sunflower fields in the Krasnodar Krai in 2011-2015

Districts	Year	Foreign hybrids of sunflower in the field*	The number of isolates				
			total	race			
				330	710	730	**334**
Belorechensky	2011	-	11	7	2	2	0
Gulkevichsky	2011	-	28	10	9	9	0
	2015	-	64	5	18	41	0
Novokubansky	2012	-	39	1	26	12	0
Labinsky		-	12	0	9	3	0
Kushchyovsky		+	3	0	2	0	1
Korenovsky	2013	+	50	24	2	40	34
Tbilissky	2014	+	70	11	5	5	49
Slavyansky	2015	-	12	7	4	1	**0**
Novopokrovsky		+**	15	0	0	0	15**
		-	7	0	1	6	**0**
Kanevskoy		+**	17	0	0	0	17**
Pavlovsky		+**	25	0	0	0	25**

* - foreign hybrids of sunflower have been cultivated in the field in any of last 5 years (before the year of sampling): '+' – yes, '-' – no; ** - *P. halstedii* isolates were collected from the foreign hybrids with Pl_6

Russia became the second European country in which race 334 has been revealed. This race was registered for the first time at the beginning of the 2000 in France and after 2007 - in the USA and Canada (Gulya,2007; Delmotte et al., 2008; Viranyi et al., 2015).

It is possible that race 334 was introduced into our country with the seeds of foreign sunflower hybrids. On the other hand, its appearance could become the result of evolutionary processes in local *P. halstedii* population, exerted by cultivating of resistant sunflower hybrids and elevated by crop rotation violations.

Experience of different countries showed that after the appearance of new races in the population of this parasite, the emergence of other races can be expected soon. As it was happened in France. After the race 100, there emerged pathotypes with virulence code 7xx which have overcome the resistance of differential lines RHA-274 (D-3): races 710 and 703 to which also lines PMI3/DM-2 (D-4) and HA-R4 (D-7) + HA-R5 (D-8) (respectively) are susceptible. Then, due to the massive deployment of new resistance genes (as Pl_6 and Pl_7), there were formed new races, that able to overcome resistance of differential lines RHA-265 (D-2) – races 3*xx*, PM-17 (D-5) – races *x*3*x*, HA-335 (D-9) – races *xx*4 и *xx*7 (Tourvieille de Labrouhe et al., 2005; Delmotte et al., 2008; Viranyi et al., 2015).

In the south of Russia after race 100, races with virulence code 3x0 (300, 310 and 330) appeared, then – 7x0 (700, 710 and 730) and the last to date – race 334. The pathotypes with virulence to the genes of resistance in differential lines 803-1 (D-6), HA-R4 (D-7) and HA-R5 (D-8) still were not recorded here.

Thus, the racial composition of the *P. halstedii* population in the south of Russian Federation has changed due to the cultivated assortment of sunflower. Races 330, 710 and 730, which dominated last years, still were widespread but their parts in the parasite population decrease. In 2012, for the first time in Russia, the race 334 has been found. It is quickly distributes and has occupied a dominant position in some fields. Further spread and accumulation of *P. halstedii* race 334 and the emergence of new pathogen pathotypes in the southern regions of Russia are predicted.

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