Maria Iwebor*, Tatiana Sergeevna Antonova and Svetlana Saukova

Changes in the Racial Structure of *Plasmopara halstedii* (Farl.) Berl. et de Toni Population in the South of the Russian Federation

DOI 10.1515/helia-2016-0008
Received April 27, 2016; accepted May 20, 2016; previously published online June 16, 2016

Abstract: The population of oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni (sunflower downy mildew pathogen) has been monitored in Krasnodar and Rostov regions and the Republic of Adygea for more than 15 years. Prior to the beginning of the 2000s there were races 100, 300, 310 and 330 in the regions. In the period from 2004 to 2007 races 100, 300, 310 and 700 were recorded 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 have been 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 region. In the period of 2013–2015 increased distribution of the race 334 in the Krasnodar region and the 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.

Keywords: downy mildew, Plasmopara halstedii, races, sunflower

***Corresponding author: Maria Iwebor,** The Department of Sunflower (Laboratory of Immunity and Molecular Labeling), Federal State Budgetary Scientific Institution «All-Russia Research Institute of Oil Crops by V. S. Pustovoit» (VNIIMK), 17 Filatov Street Krasnodar 3500038, Russian Federation, E-mail: maria-iwebor@mail.ru
Tatiana Sergeevna Antonova, Svetlana Saukova, The Department of Sunflower (Laboratory of Immunity and Molecular Labeling), Federal State Budgetary Scientific Institution «All-Russia Research Institute of Oil Crops by V. S. Pustovoit» (VNIIMK), 17 Filatov Street Krasnodar 3500038, Russian Federation

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 southern regions of the Russian Federation (Krasnodar and Rostov regions and the Republic of Adygea) for 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 breeding became affected in the Krasnodar region (Tikhonov and 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 sunflower seeds for sowing of foreign breeding were freely delivered into the country and shortening in terms of crop return in fields 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 regions 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 the Russian Federation (Krasnodar and Rostov regions and the Republic of Adygea).

Materials and methods

The leaves from the infected by downy mildew sunflower plants were collected from the fields in the Adigeya republic, Krasnodar and Rostov regions 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 sunflower variety VNIIMK 8883.

Seeds of differentials were placed for germination in rolling up filter paper at the temperature 25 °C (Figure 1: steps 1 and 2). After 60–72 h, at the radicles length 1.0–2.0 cm (Figure 1: step 3), the germinated seeds (hereinafter referred to as "seedlings") of differential lines were cleared of husk and laid down by rows in growth trays with wet sterilized sand covered by filter paper (10 seedlings of each line per one). The radicles of seedlings were covered by wet cotton wool. 150 ml of zoosporangial suspension of isolates (concentration about 10^6 zoosporangia/ml) was added into growth trays (one *P. halstedii* isolate per a tray) and incubated during 16–20 h at the temperature 16 °C (Figure 1: step 4). Inoculated sunflower plants were grown at the temperature 25 ± 2 °C (16 h photoperiod) (Figure 1: step 5) and after 7–9 days (depending on a dates of first true leaves appearance at seedlings) were placed in darkness, at 16 °C and 100 % humidity, for 16–20 h to induce *P. halstedii* sporulation (Figure 1: step 6). Plants with sporulation on leaves or with abundant sporulation only on cotyledons were classified as susceptible (Figure 1: step 7). If some of differential lines displayed partial infection, these lines were re-inoculated using *P. halstedii* sporulation from the universal susceptible or doubtful lines. If some of differential lines displayed partial infection, these lines were re-inoculated, using *P. halstedii* sporulation from the universal susceptible or doubtful lines.

Results and discussion

Until 2007 in the southern regions of Russia (Krasnodar and Rostov regions and the Republic of Adygea) seven races of *P. halstedii* were found. Among them, during 2004–2007, races 100, 300, 310 and 700 formed together about 2,5 %, and and races 330, 710 and 730 (about 65, 13.5 and 19 %, respectively) were the most common.

Ever since, 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 races in the regions in different years.

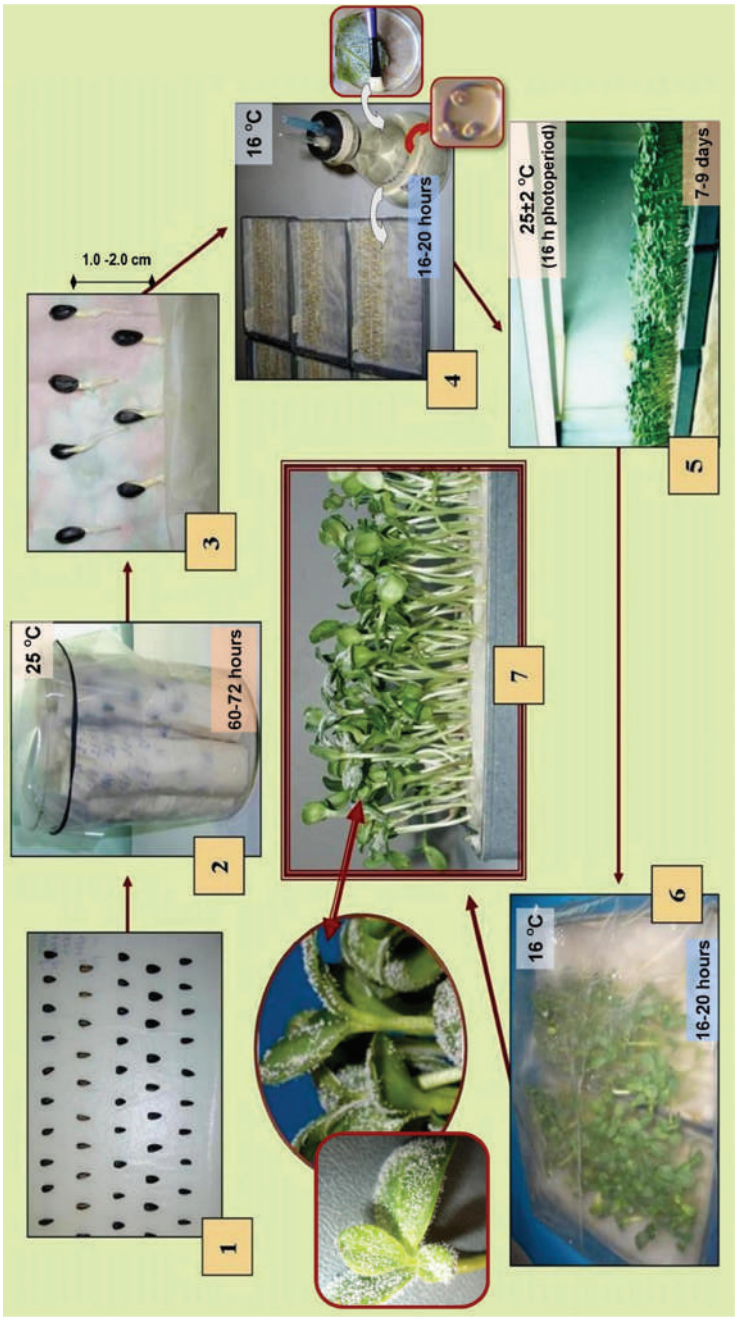

Figure 1: Identification of the *Plasmopara halstedii* races (schematically): 1 – seeds of differential lines are placed for germination on wet filter paper; 2 – rolls with germinating seeds; 3 – germinated sunflower seeds; 4 – germinated seeds of differential lines are cleared of husk, placed by rows into growth trays and inoculated (one isolate of *P. halstedii* per a tray); 5 – cultivation of infected seedlings of sunflower differential lines; 6 – growth trays with infected seedlings into wet chamber; 7 – sunflower seedlings with sporulation.

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	R	Rfi	F	R	Rfi	F	R	Rfi	F	R	Rfi
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

Notes: * F – the frequency of the race occurrence in the fields, %; R – race proportion in the total number of identified *P. halstedii* isolates, %; Rfi – minimum and maximum percent (%) of the race in positive samples.

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

Races 100, 300 and 700 were not found after 2007. However, oospores of *P. halstedii* are capable 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 regions like 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 proportion in the racial structure of 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, the race 330 has been found on plants of common ragweed (*Ambrosia artemisiifolia* L.) in Krasnodar region in different years (2011, 2013 and 2015). All isolates collected by us from common ragweed plants belonged only to this race. Analyze of SNP DNA loci proved identity of these isolates and isolates of the race 330 from sunflower (Iwebor *et al.*, 2012). Thus, the race 330 can persist in the local population of *P. halstedii* on common ragweed. 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 most of separate agrocoenosises.

In 2012 one isolate of race 334 was discovered in the Krasnodar region. For the first time in Russia, there was detected a pathotype able to overcome the resistance gene Pl_6. In 2013 the race 334 has been found again in one field. In 2014–2015 increased distribution of this race was observed in the Krasnodar region and the Republic of Adygea (Table 3). It was present in almost a half of the 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 the 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.

In one of the sunflower fields in the Rostov region (2014), race 330 dominated and race 310 was 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

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

Districts	Year	Foreign hybrids of sunflower in the field*	The number of isolates					
			Total	Races				
				310	330	710	730	334
Rostov region								
Azovsky	2011	–	19	0	6	6	7	0
	2014	–	55	0	40	7	8	0
		–	64	11	53	0	0	0
The Republic of Adygea								
Giaginsky	2012	–	28	0	7	11	10	0
	2014	+	10	0	0	5	2	**3**
Shovgenovsky	2015	+	16	0	0	3	3	**10**

Note: * – foreign sunflower hybrids have been cultivated in the field in any of last five years (before the year of sampling): "+" – yes, "–" – no.

Table 4: Races of *P. halstedii*, found in the sunflower fields in Krasnodar region in 2011–2015.

Districts	Year	Foreign hybrids of sunflower in the field*	The number of isolates				
			Total	Races			
				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**

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

varieties were cultivated there. In the other two fields of this region and in one of the fields in the Adygea republic (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 the Krasnodar region (Table 4). In the fields, where only domestic varieties and hybrids have been cultivated (at least five last years before the year of sampling), races 330, 710 and 730 were isolated, but not the race 334.

The race 334 was revealed in the field where in any of last five years (before the year of sampling) foreign sunflower hybrids have been cultivated. For example, they are 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.

Russia became the second European country where the 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 (Delmotte *et al.*, 2008; Viranyi *et al.*, 2015).

Possibly, 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, caused and stimulated 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. The same situation was observed 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, 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 and *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 the Russian Federation has changed due to the cultivated assortment of sunflower. Races 330, 710 and 730, dominated last years, still were widespread but their proportions in the parasite population decrease. In 2012, for the first time in Russia, the race 334 has been found. It has been quickly distributing and has occupied the 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.

References

Antonova, T., 2003. Selekcia podsolnechnika na immunitet. In: Istoriya nauchnih issledovaniy vo VNIIMK. Krasnodar, pp. 253–272. (in Russian)

Antonova, T., Iwebor, M., Araslanova, N., 2008. Races of *Plasmopara halstedii* on sunflower in separate agrocenoses of Adigeya Republic, Krasnodar and Rostov regions in Russia. Proc. of 17th Int. Sunflower Conf. Cordoba, Spain 1: 85–90.

Delmotte, F., Giresse, X., Richard-Cervera, S., M'baya, J., Vear, F., Tourvieille, J., Walser, P., Tourvieille de Labrouhe, D., 2008. Single nucleotide polymorphisms reveal multiple introductions into France of *Plasmopara halstedii*, the plant pathogen causing sunflower downy mildew. Infection, Genetics and Evolution 8: 534-540. doi: 10.1016/j.meegid.2008.02.012.

Iwebor, M.V., Ramazanova, S.A., Antonova, T.S., 2012. Race identification of *Plasmopara halstedii* isolates from infected plants of *Ambrosia artemisiifolia*. Oil Crops, Scientific and Technical Bulletin of VNIIMK 2: 163–166. in Russian.

Tikhonov, O.I., Zaychuk, V.F., 1984. Novaya rasa lozhnoy muchnistoy rosy. Oil crops, p. 20. (in Russian)

Tourvieille de Labrouhe, D., Gulya, T., Maširevic, S., Penaud, A., Rashid, K., Viranyi, F., 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). Proc. of the 15th Int. Sunflower Conf., Toulouse, France, I 61–66.

Tourvieille de Labrouhe, D., Mestries, E., Walser, P., 2005. Quelles perspectives pour la lutte ge´ne´tique vis-a` -vis du mildiou du tournesol? Ole´agineux Corps gras Lipides 12: 85–93.

Viranyi, F., Spring, O., 2011. Advances in sunflower downy mildew research. European Journal of Plant Pathology 129: 207–220.

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