# Maria Iwebor\*, Tatiana Antonova and Svetlana Saukova Occurrence and Distribution of Races 713, 733 and 734 of Sunflower Downy Mildew Pathogen in the Russian Federation

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**Abstract:** The samples of *Plasmopara halstedii* (the causal agent of sunflower downy mildew) were collected on sunflower fields in south of the Russian Federation: in the Krasnodar, Rostov and Stavropol regions, and in the Republic of Adygea in 2016 and 2017. Virulence code of 545 isolates was identified; i. e. 280 isolates originating from 24 fields in 2016, and 265 isolates from 17 fields in 2017. Races 330, 334, 710 and 730, widespread within the region in previous years, were found in all mentioned areas. In addition, races 713, 733 and 734 have been detected in several districts of the Krasnodar region since 2016. This is the first report of these races in Russia and above that the first record throughout Europe and Asia for the races 733 and 734. The identification of new *P. halstedii* races was confirmed by the method of cross inoculations from individual differential lines. The phenotype corresponding to virulence code 734 was in some cases disclosed as a mixture of race 334 with 710 or 730. The presence of several *P. halstedii* races on an individual plant was also confirmed for the first time in the territory of the Russian Federation.

**Keywords:** causal agent, oomycete, population, *Plasmopara halstedii*, race, virulence code

## Introduction

Downy mildew is one of the most widespread and harmful diseases of sunflower in many countries of the world, including the Russian Federation (RF). The causal agent of the disease is a biotrophic oomycete *Plasmopara halstedii* 

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(Farl.) Berl. et de Toni. The pathogen has high evolutionary potential: it develops new races that overcome the action of sunflower resistance genes. In the 1970s only two races of the parasite were known in the world (Zimmer, 1974), by 2018 more than 45 races were recorded (Bán *et al.*, 2018; Sedlářová *et al.*, 2016; Spring and Zipper, 2018; Viranyi *et al.*, 2015). Within RF, changes in the population of *P. halstedii* have been studied in the south of the country, in the areas of the Krasnodar and Rostov regions and the Republic of Adygea over last 20 years. Until 2016, eight races of *P. halstedii* were recorded. Recently, the most widespread have been races 330, 710, 730 and 334 (Iwebor *et al.*, 2016).

The aim of the research was the identification of three-digit virulence codes for *P. halstedii* isolates originating in the south of RF using a standard set of nine differential lines of sunflower (Tourvieille de Labrouhe *et al.*, 2000a).

## Materials and methods

# The collection of P. halstedii isolates and identification of races

In June and July of 2016 and 2017 leaves with downy mildew sporulation were collected from systemically infected sunflower plants in fields in Krasnodar, Rostov and Stavropol regions, and in the Republic of Adygea (RF).

Part of the freshly collected samples were used to determine the pathogen races, other samples were stored in a freezer ( $-80 \text{ }^{\circ}\text{C}$ ) for subsequent identification.

*P. halstedii* races were identified by a laboratory method using a set of nine differential lines according to Tourvieille de Labrouhe *et al.* (2000a):

triplet-group 1: D-1 – VNIIMK 8883 (the universally susceptible cultivar instead of line HA-304), D-2 – RHA-265, D-3 – RHA-274;

triplet-group 2: D-4 - PMI-3, D-5 - PM-17, D-6 - 803-1;

triplet-group 3: D-7 - HA-R4, D-8 - QHP-1, D-9 - HA-335.

The seeds of differentials were germinated at a temperature of 25 °C in rolls of filter paper to a root size of 1–2 cm. For 10–15 germinated seeds (hereinafter referred to as "seedlings") of each differential line were cleared of husk and laid down by rows in plastic growth trays with wet calcined river sand covered by filter paper. The roots were covered with strips of moist cotton wool and filter paper. To prepare the inoculum, *P. halstedii* sporulation was carefully rinsed from the infected leaves collected in the field (fresh or frozen) in settled tap water. 150 mL of inoculum, i. e. suspension of zoosporangia  $(1 \times 10^6 \text{ in mL}^{-1})$  was added into growth trays (one *P. halstedii* isolate per a tray) and incubated for 14–20 h at 16–20 °C. Seedlings were grown until the appearance of the first pair of

true leaves (0.5–1 cm in size) at a temperature of 22-25/16-18 °C and 16/8 h day/ night. After seven to nine days, the growth trays were abundantly watered with settled tap water, covered with polyethylene film to create a moist chamber and placed overnight in darkness at 16–20 °C to induce *P. halstedii* sporulation. For the race identification, the presence of sporulation and its intensity on the first pair of true leaves and cotyledons was taken into account, as defined by Tourvieille de Labrouhe *et al.* (2000a). Plants without sporulation or with weak sporulation only on cotyledons were identified as resistant. Susceptibility was recorded by the presence sporulation on leaves or intensive sporulation on cotyledons only.

#### Confirmation of virulence codes of new races

Cross re-inoculations of susceptible sunflower differential lines (depending on the *P. halstedii* race) were conducted (Table 1). For this, two to three seedlings with sporulation per certain differential line with sporulation were placed separately into sterile Petri dishes on moist filter paper and left overnight at the temperature of 16–20 °C to develop a new sporulation of *P. halstedii*. Fresh sporulation from each seedling individually has been used to inoculate the universally susceptible cultivar VNIIMK 8883 and differential lines, susceptible to the researched race (15–20 seedlings of each line) (Table 1). During the testing of race 713, it was used sporulation from single seedlings of lines HA-R4 or QHP-1 to inoculate VNIIMK 8883 and lines RHA-274, HA-R4 and QHP-1. For race 733, the same procedure has been applied but PM-17 was added to the mentioned differentials. For race 734, it was used sporulation from single seedlings of lines RHA-274 or HA-335 to inoculate VNIIMK 8883 and lines RHA-274, PM-17 and HA-335.

The resistant/susceptible reaction was taken into account as described above. When it was doubtful, the procedure was repeated until the indisputable results.

<i>Plasmopara halstedii</i> races		Sunflower differentials
	source of infection	inoculated
713	HA-R4 or QHP-1	VNIIMK 8883, RHA-274, HA-R4, QHP-1
733	HA-R4 or QHP-1	VNIIMK 8883, RHA-274, PM-17, HA-R4, QHP-1
734	RHA-274 or HA-335	VNIIMK 8883, RHA-274, PM-17, HA-335

**Table 1:** Sunflower differentials used in cross inoculations during testing of new

 *Plasmopara halstedii* races.

At the final stage, the whole set of nine sunflower differentials for each isolate was infected, using sporulation from one seedling of a differential line. For races 713 and 733 – from HA-R4 (D-7) or QHP-1 (D-8), for race 734 – from RHA-274 (D-3) or HA-335 (D-9).

## Results

Virulence code was determined for 545 isolates of *P. halstedii*, collected in 2016 and 2017 at sunflower fields in the Krasnodar, Stavropol and Rostov regions and the Republic of Adygea; i. e. 280 isolates from 24 fields in 2016 and 265 isolates from 17 fields in 2017. These were large production fields, except for the experimental fields of VNIIMK in city Krasnodar. As a result of the analysis of *P. halstedii* samples collected in 2016 and 2017, previously determined races were identified. In Stavropol and Rostov regions races 330, 710 and 730 were found. The same races and race 334 were identified in the Republic of Adygea and in most fields of the Krasnodar region. In addition to them, in 2016, in some fields of the Krasnodar region three *P. halstedii* races were found in RF for the first time, i. e. 713, 733 and 734. Table 2 shows the susceptibility of sunflower differentials by these new and other races that were found in the region in 2016 and 2017.

Sunflowe	er differenti	als			Plasm	opara hal	stedii races	5	
			330	334	710	730	newly	recordec	l in RF
	number	Pl					713	733	734
VNIIMK 8883	D1	no <i>Pl</i>	S	S	S	S	S	S	S
Rha-265	D2	$Pl_1$	S	S	S	S	S	S	S
Rha-274	D3	Pl <sub>2/21</sub>	R	R	S	S	S	S	S
PMI-3	D4	Pl <sub>PMI3</sub>	S	S	S	S	S	S	S
PM-17	D5	Pl <sub>5</sub>	S	S	R	S	R	S	S
803-1	D6	Pl <sub>5+</sub>	R	R	R	R	R	R	R
HA-R4	D7	$Pl_{15}$	R	R	R	R	S	S	R
QHP-1	D8	<i>Pl</i> <sub>1/15</sub>	R	R	R	R	S	S	R
HA-335	D9	$Pl_6$	R	S	R	R	R	R	S
HA-337		$Pl_7$	R	S	R	R	R	R	S
Rha-340		Pl <sub>8</sub>	R	R	R	R	R	R	R
Rha-419		Plarg	R	R	R	R	R	R	R

**Table 2:** The susceptibility of sunflower differentials by *Plasmopara halstedii* races identified inthe Russian Federation in 2016 and 2017.

Phenotype of sunflower differential reaction to *P. halstedii* infection: S-susceptible; R-resistant. Information on resistance genes (*Pl*) as reported by Gascuel *et al.* (2015) and Viranyi *et al.* (2015)

All newly recorded races affected universally susceptible cultivar VNIIMK 8883 and differential lines Rha-265, Rha-274 and PMI-3. Line PM-17 was susceptible to races 733 and 734. Races 713 and 733 affected lines HA-R4 and QHP-1. Line HA-335, containing resistance gene  $Pl_6$ , was susceptible to race 734. Differential line 803-1 was resistant to all local pathogen races.

For resistance to races 713, 733 and 734 were also tested differential lines HA-337 ( $Pl_7$ ), HA-340 ( $Pl_8$ ) and RHA-419 ( $Pl_{arg}$ ). Differentials HA-340 and RHA-419 were resistant to these three new races as to all other local races. Line HA-337 was resistant to races 713 and 733 but susceptible to race 734 (as to race 334).

The virulence codes of newly found races were confirmed through multiple cross re-inoculations of susceptible differential lines of sunflower. During the testing of race 734 by the method of cross re-inoculations it was established that, in addition to isolates of real race 734, in many agrocenoses this virulence code was the result of mixing of *P. halstedii* races: 334 + 710 and 334 + 730 (Table 3).

The prevalence of races 713, 733 and 734 in the areas of the Krasnodar region in 2016 and 2017 is shown in the Table 3 and Figure 1. In 2016, race 713 was identified at four surveyed fields; races 733 and 734 were identified at five fields. In 2017, race 713 was identified at four fields, race 733 -at three fields, race 734 at two fields. In most cases, races 713 and 733 were simultaneously present at the same field. The situation was similar with the race "734": at the same fields in most cases the pathogen was present as a true race 734 and as a mixture of races 334 + 710 or 334 + 730 in one plant. Races 733 and 734 were identified at the same field in 2016 in the Kurganinsky district. In 2016, races 713, 733 and 734 comprised from 4 to 100% of isolates samples, in 2017 – from 9 to 100%. In some samples of isolates, along with new races, races widespread in a region for a long time were present: most often races 710 and 730, less often races 330 and 334.

## Discussion

The monitoring of *P. halstedii* population in the south of the RF was carried out for many years. It showed that the distribution of races 713, 733 and 734 in the region differs from the spread of the previous new race - 334. In 2012, the first and only isolate of race 334 was found in the Krasnodar region, in 2013 - single isolates at two fields. Since 2014, this race has been identified in most areas of the Krasnodar region and in the Republic of Adygea (Iwebor *et al.*, 2016). Races 713, 733 and 734 were present at several fields in remote areas of the region from the first year of detection (2016), ranging from 3.8 to 100% of isolates (Table 3).

1 representation of <i>Plasmopara halstedii</i> races 713, 733 and 734 in popu 2016 and 2017.	lations at the different sunflower fields in the	
entation of <i>Pl</i> nd 2017.	3 and 734 in popu	
	entation of Pl	2016 and 2017.

Locality	Collection vear	N° of field	The number of isolates				Plasmopa	Plasmopara halstedii races <sup>1</sup>	es <sup>1</sup>			
						newl	newly recorded			others	ers	
				713	733		«734» <sup>2</sup>		330	334	710	730
						734	710 + 334	730 + 334				
		1	11	64	36	0	0	0	0	0	0	0
•	2016	2	16	75	25	0	0	0	0	0	0	0
Novopokrovsky		ſ	12	0	0	59	8	33	0	0	0	0
	2017	4	12	100	0	0	0	0	0	0	0	0
		5	6	11	11	0	0	0	0	0	78	0
		1	24	46	54	0	0	0	0	0	0	0
	2016	2	14	71	29	0	0	0	0	0	0	0
Krylovskoy		m	12	0	100	0	0	0	0	0	0	0
	2017	4	10	80	20	0	0	0	0	0	0	0
		5	23	17	5	0	0	0	0	0	52	26
Kurganinsky	2016		30	0	7	30	0	10	33	0	m	17
Labinsky	2016		18	0	0	44	11	9	11	0	0	28
Korenovskv	2016		9	0	0	100	0	0	0	0	0	0
	2017		12	0	0	100	0	0	0	0	0	0
	2016		80	0	0	17	4	8	∞	17	19	27
Nidoliudal	2017		45	0	0	6	4	7	6	11	24	36



**Figure 1:** Areas of detection of *Plasmopara halstedii* races 713, 733 and 734 in the Krasnodar region (Russian Federation) during 2016 and 2017.

Race 734 is the second race in Russia after race 334, which is able to overcome the resistance of  $Pl_6$  gene. Earlier, race 734 was discovered in the United States of America on a cultivated sunflower (Gulya and McMullen, 2011; Viranyi *et al.*, 2015). In addition, this race was identified there on *Rudbeckia fulgida* (Rivera *et al.*, 2016). The presence of race 734 had been assumed in Hungary but attempts to confirm it experimentally failed (Bán *et al.*, 2016). Until now, according to the information available to us, race 734 was not recorded in other countries, except the United States.

Races 713 and 733 are the first races of *P. halstedii* in the RF that are able to overcome the resistance genes in differential lines HA-R4 ( $Pl_{15}$ ) and QHP-1 ( $Pl_{1/15}$ ) (resistance genes as reported by Gascuel *et al.* (2015)). Like race 734, both of these races were previously identified in the USA. Before 2007, race 713 has also occurred in Spain (Virányi and Wacgz, 2000), Serbia (Gulya, 2007) and Turkey (Viranyi *et al.*, 2015), and between 2012–2015 in Argentina (Bazzalo *et al.*, 2016). Prior to that, race 733 was spread only in the North America, in the USA (since 2005) (Gulya, 2006) and Canada (Rashid, 2009).

The fact of occurrence of new *P. halstedii* races in RF was confirmed by additional inoculations of certain differential lines (Table 1). It was especially important for proving the novelty of race 734, since this virulence code could be obtained due to the mixture of races, which existed in the region earlier, in one sample: 334 + 7x0 (700, 710 or 730). Cross inoculations proved the validity of doubts: in addition to pathogen isolates representing real race 734, it was identified the simultaneous presence of races 334 and 710 or 730 in the same plant (Table 3). Many foreign researchers have written about the possibility of the mixture of different *P. halstedii* races within a single isolate (Intelmann and Spring, 2002; Rozynek and Spring, 2000; Trojanová *et al.*, 2017; Virányi and Gulya, 1995), but until now it has not been reported for the parasite population on the territory of RF.

It is possible that all these races, new for Russia, have entered the country with the seeds of sunflower hybrids from abroad. At the beginning of the twentieth century, Young and Morris (1927) expressed the opinion that the pathogen migrates from continent to continent with the seeds. It was confirmed later (Cohen and Sackston, 1974; Döken, 1989; Ioos et al., 2007; Novotel'nova, 1966; Zimmer, 1975). The results of studies have shown that seeds containing mycelium or even oospores of *P. halstedii* rarely produce plants with typical symptoms of the disease (Cohen and Sackston, 1973; Döken, 1989; Leppik, 1962; Tourvieille de Labrouhe et al., 2000b). However, in 2016 and 2017, in many areas of the Krasnodar region, weather conditions were favorable for the development of *P. halstedii* (abundant precipitation at low air temperatures in the first half of the growth period). This could contribute to the manifestation of symptoms of the disease, which started as primary/systemic infection. Still, these races could also develop during the evolutionary processes taking place in the pathogen population in the Krasnodar region. The detection of a large number of sunflower plants infected with race "734", representing in fact a mixture of oomycete races (Table 3), is in favor of the latter. As Spring (2000), Spring and Zipper (2006) and Delmotte et al. (2008) pointed out, the presence of thallomes of different P. halstedii races in one plant increases the probability of occurrence of races as a result of a sexual process or asexual recombinations. Therefore, there is a high probability of the emergence of new races of pathogen in the Krasnodar region and other regions of RF as a result of evolutionary processes and/or introduction with seeds. We guess that the races 713, 733 and 734 will soon expand their spreading area.

### Conclusions

For the first time on the territory of the Russian Federation, races 713, 733 and 734 of the sunflower downy mildew causal agent were discovered. To our

knowledge, this is the first report of races 733 and 734 throughout Europe and Asia. By the method of multiple cross re-inoculations from individual differential lines, the simultaneous presence of *P. halstedii* races with different virulence code (334 together with 710 or 730) in one sunflower plant was found. The fact of mixture of races of this parasite in one plant was not previously established in the country. At the present time in the Russian Federation the pathogen has overcome the resistance mechanisms of many *Pl* genes, including *Pl*<sub>6</sub>, *Pl*<sub>15</sub> and *Pl*<sub>1/15</sub>, but can be controlled by *Pl*<sub>5+</sub> (in sunflower differential line 803-1), *Pl*<sub>8</sub> (in Rha-340) and *Pl*<sub>arg</sub> (in Rha-419). The high diversity of virulence within the *P. halstedii* populations indicates the need for its continuous monitoring to limit pathogen transmission.

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