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The first report of *Plasmopara halstedii* race 337 in the Russian Federation

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Abstract: Sunflower downy mildew caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni is a destructive and widespread disease. More than 50 races of *P. halstedii* have been recorded worldwide. In 2020, in the Russian Federation (Zernogradsky district, Rostov region), a globally new race 337 was identified for the first time. The pathogen was identified on the plants of a foreign sunflower hybrid bearing the resistance gene Pl_6. According to the five-digit racial nomenclature, its virulence profile was determined as 337 *53*. It is the first *P. halstedii* race recorded in the Russian Federation that simultaneously infects all differential lines of the 3rd triplet, i.e., HA-R4, HA-R5 and HA-335. The sunflower lines RHA-274, 803-1, PSC8, RHA-419 and RHA-340 were resistant to it. All the collected isolates of the new race were susceptible to the fungicide mefenoxam.

Keywords: *Helianthus annuus*; mefenoxam; oomycete; resistance; sunflower downy mildew; virulence

Downy mildew is one of the most widely spread and harmful diseases of sunflowers (*Helianthus annuus* L.) in many countries, including the Russian Federation (Spring 2019). The disease is caused by an obligate parasite *Plasmopara halstedii* (Farl.) Berl. et de Toni. This oomycete, like its main host *H. annuus*, is considered to have originated in North America (Novotelnova 1966). From there, it was spread to other continents with sunflower seeds (Gulya 2007), in which it can be preserved in the form of mycelium and oospores (Spring & Zipper 2000). In Russia, sunflower downy mildew was first registered in 1947 and, in the early 1950s, it became epiphytotic (Novotelnova 1966).

The basic protective measures against sunflower downy mildew are agrotechnical methods, fungicide seed treatments and genetic resistance. For many years phenylamide systemic fungicides, metalaxyl, and later its isomer, mefenoxam (synonym metalaxyl-M), have been widely used to manage downy mildew. However, the resistance of *P. halstedii* to metalaxyl has been registered since the 1980s (Oros & Virányi 1984) and to mefenoxam – since the early 2000s (Molinero-Ruiz et al. 2008). The resistance of *P. halstedii* to mefenoxam has also been reported in Russia (Iwebor et al. 2019).

Genetic resistance of the sunflower to *P. halstedii* is provided by dominant resistance genes, named *Pl* genes, of which 36 (Pl_1–Pl_{35}, and Pl_{arg}) have been identified up to 2019 (Ma et al. 2019). The search for new sources of resistance remains relevant because the pathogen has already overcome resistance genes: about 50 physiological races of *P. halstedii* have been registered worldwide (Viranyi et al. 2015; Spring 2019; Gilley et al. 2020; Miranda-Fuentes et al. 2021) and 11 of them were known to occur in Russia until 2020 (Iwebor et al. 2019). In this article, we report on an undescribed race 337, isolated from sunflower plants infected with downy mildew in 2020 in the southern part of the Russian Federation.

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MATERIAL AND METHODS

In June 2021, plants of a foreign commercial sunflower hybrid (Pl_6) with symptoms of systemic infection of downy mildew were collected from a 70 ha field in the Zernogradsky district of the Rostov region, the Russian Federation. In the previous crop rotation of sunflowers, an unknown foreign hybrid was grown in the field four years previously. The affected plants were stunted, and their leaves were chlorotic, containing white sporulation on the lower surface of the leaf (Figure 1A). One plant was considered as one pathogen isolate. The collected leaves were washed with tap water and placed separately in a humid chamber at 20 °C overnight to obtain fresh sporulation of the pathogen.

The morphology of the pathogen as well as release of zoospores from zoosporangia in water was observed with a Motic BA-310 Digital Compound Microscope (MoticEurope, S.L.U., Spain) coupled to a digital camera. *P. halstedii* as the pathogen of sunflower plant was confirmed based on the morphology of the sporangiophores and sporangia (Hall 1989) (Figures 1B and 1C).

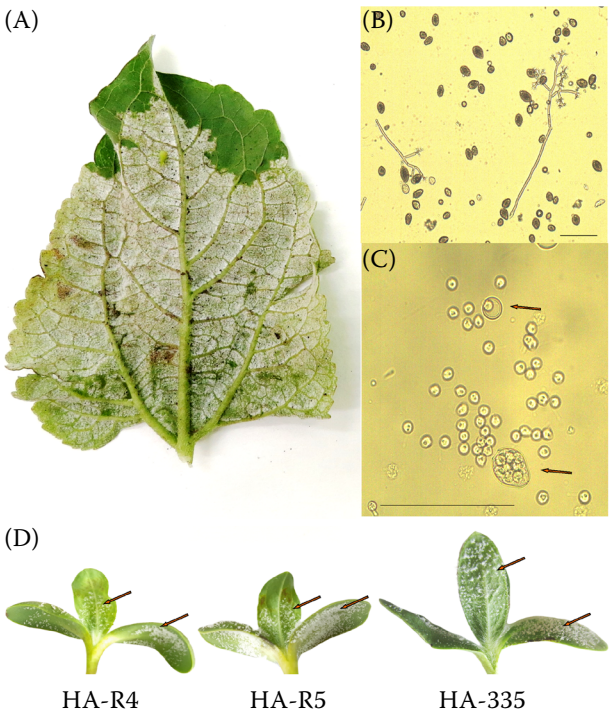

Figure 1. *Plasmopara halstedii* (Farl.) Berl. et de Toni (A) sporulation on the lower surface of a leaf of a systemically infected sunflower plant, (B) zoosporangiophores and zoosporangia, (C) zoosporangia (marked by arrows) releasing zoospores, (D) the sporulation (marked by arrows) on the cotyledons and first true leaves of the sunflower lines from the third triplet of the differential set Bars = 100 µm (B, C)

Determination of the race belonging to the 27 isolates was carried out using a set of nine differential lines of sunflowers according to the internationally standardised nomenclature (Tourvieille de Labrouhe et al. 2000) (Table 1), by whole seedling inoculation as described by Iwebor et al. (2016). The phenotypic reaction of the differential lines to the *P. halstedii* infection was determined by the presence of sporulation and its intensity on the cotyledons and the first pair of true leaves of the sunflower plants according to Tourvieille de Labrouhe et al. (2012). Each *P. halstedii* isolate was retested. For this purpose, one sporulated seedling per the lines HA-R4, HA-R5, and HA-335 was used to inoculate the nine sunflower differential lines from the standard set again. Additionally, the same inoculum was used to inoculate the lines DM-2 and HA-R5 [identical to the lines PMI-3 (D4) and QHP-1 (D8), respectively] (Table 1) as proposed by Gulya (1995). Eleven isolates were tested for the third time to determine the five-digit virulence code (race) using a set of 15 differential lines, as proposed by Tourvieille de Labrouhe et al. (2012). The lines HA-337 and RHA-340 were supplemented too (Table 1).

The sensitivity of the isolates to the fungicide mefenoxam was tested using the method described by Albourie et al. (1998). The fresh sporulation of each isolate was washed separately and the concentration was adjusted to 105 zoosporangia per ml of water. Eleven isolates were tested separately and 16 were combined (their inocula were mixed in equal proportion). Sunflower seeds of the cultivar VNIIMK 8883 were treated with Apron XL 350 FS (350 g/L mefenoxam, Syngenta, Switzerland) with the registered rate in Russia (3 mg/kg seeds), and untreated seeds were used as the control.

RESULTS AND DISCUSSION

Each of the 27 *P. halstedii* isolates infected all the differential lines of the standard set, except for the lines RHA-274 and 803-1, therefore, race 337 was identified (Table 1). Since the field isolates can be a mixture of several pathotypes, single zoosporangia or zoospore isolates can be used for the precise race identification. However, such isolations are routine and ineffective (the success

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Table 1. The differential lines of the sunflowers and the reaction phenotype of the new race of *Plasmopara halstedii* (Farl.) Berl. et de Toni

Triplet number	Sunflower differential line[1]	Resistance genes[2]	Virulence value	Reaction phenotype	Virulence code/race
Standard set of differential lines					
1	cultivar VNIIMK 8883	none	1	S	3
	RHA-265	Pl_1	2	S	
	RHA-274	Pl_2, Pl_9, Pl_{10}, Pl_{11}, Pl_{12}, Pl_{21}	4	R	
2	PMI-3	Pl_{22}	1	S	3
	DM-2	Pl_5, Pl_{11}, Pl_{12}	1	S	
	PM-17	Pl_{5-}	2	S	
	803-1	Pl_{5+}, Pl_{803}	4	R	
3	HA-R4	Pl_{12}, Pl_{14}, Pl_{16}	1	S	7
	QHP1	Pl_1, Pl_{13}	2	S	
	HA-R5	Pl_{13}	2	S	
	HA-335	Pl_6	4	S	
Proposed set of differential lines					
4	YVQ-B (instead of Y7Q)	Pl_{6-}	1	S	5
	PSC8	Pl_2	2	R	
	HIR-34 (black seeds; instead of XA)	Pl_4	4	S	
5	83HR4RM (instead of PSS2RM)	Pl_7	1	S	3
	XRQ-B (instead of VAQ)	Pl_{5+}	2	S	
	RHA-419	Pl_{arg}	4	R	
Supplemental lines					
HA-337		Pl_7	–	S	–
RHA-340		Pl_8	–	R	–

S – susceptible; R – resistant according to Tourvieille de Labrouhe et al. (2012)

[1]Standardised set according to Tourvieille de Labrouhe et al. (2000), proposed set according to Tourvieille de Labrouhe et al. (2012); [2]the data collected in the publication of researchers for 2019 and 2020 (Pecrix et al. 2019; Ramazanova & Antonova 2019; Ma et al. 2020)

rate is about 1–2%), and alternate methods are practiced (Trojanová et al. 2017). We retested each *P. halstedii* isolate using the sporulation from the individual seedlings of the lines HA-R4, HA-R5, and HA-335 to inoculate the nine differential lines from the standard set. The results confirmed that all the isolates belonged to race 337. For all the isolates, the number of zoospores produced per sporangium ranged from 17 to 51 and was predominantly 27–29 on average.

Race 337 is the first one registered in Russia that simultaneously infects all the differential lines of the 3rd triplet, i.e., HA-R4, HA-R5 and HA-335 (Figure 1D). Previously, races infecting these lines have been reported in the United States (race 737), France (races 307, 707, and 717) (Viranyi et al. 2015), and Spain (race 317) (Miranda-Fuentes et al. 2021). To our knowledge, this is the first report of race 337 in the world population of *P. halstedii*.

Using an extended set of 15 differential lines (Tourvieille de Labrouhe et al. 2012), which have yet to be internationally approved (Sedlářová et al. 2016), a five-digit race code was determined for 11 isolates. Two of six additional lines (PSC8 and RHA-419) were not affected (Table 1). The five-digit code of the new race was determined as 337 *53*. Among the supplemental lines, the line HA-337 was susceptible, while the line RHA-340 was resistant. The line RHA-340 is one of three additional lines proposed by Gilley et al. (2020) to determine the six-digit virulence code. The need to expand the set of differentiators emerged after the appearance of *P. halstedii* pathotypes that overcame the action of Pl_8 in the line RHA-340 (Gilley et al. 2020;

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Martín-Sanz et al. 2020). Thus, the new race 337 *53* overcame many resistance genes, but can be controlled by some others, e.g., Pl_{803} (in line 803-1), Pl_8 (in line RHA-340), and Pl_{arg} (in line RHA-419).

Seed treatments with fungicides can protect sunflower seedlings from infection from a disease through the soil. Mefenoxam is quite effective among a limited number of fungicides against *P. halstedii*. However, the biotypes that are resistant to mefenoxam have spread worldwide (Molinero-Ruiz et al. 2008; Körösi et al. 2021). In the Russian Federation, resistance to mefenoxam was detected among isolates of the pathotypes 710, 730 and 734 (Iwebor et al. 2019). All the isolates did not show resistance to the fungicide mefenoxam: 100% of the sunflower plants in the variants with treated seeds had no signs of disease, while 100% of the sunflower plants were affected by downy mildew in the control (without any treatment).

A new race 337 was first identified on a foreign sunflower hybrid carrying Pl_6, suggesting that it could be introduced via seeds in the Russian Federation and could have spread to other countries, but has not yet been defined. Sunflower downy mildew from seed infections is considered to develop latently and rarely exhibits typical disease symptoms (Cohen & Sackston 1974). However, weather conditions favourable for the development of *P. halstedii* (high air humidity at low temperature) could contribute to the manifestation of disease symptoms. We assume that the new race was introduced with imported seeds of a foreign hybrid in a previous sunflower rotation four years ago. In this case, the disease developed latently; there was an accumulation of the infection and its reservation in the soil. Moreover, during the sunflower rotation in 2020, the soil became a source of the primary infection, which led to a systemic plant disease and the manifestation of typical symptoms. At the same time, the possibility cannot be excluded that the development of a new race in the local pathogen population as a biotype that had overcome the action of sunflower resistance genes exist in widely cultivated hybrids and cultivars of sunflowers.

The development of new races of the pathogen and their further spreading is almost unavoidable; therefore, it is necessary to carry out an annual monitoring of *P. halstedii* populations and study the traits of isolates of the new races. Such data allow selecting the best strategies for controlling the sunflower downy mildew (including the selection of cultivars and hybrids, fungicides, breeding for resistance, etc.), saving the sowings from the epiphytotic affection and obtaining maximum yields.

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