Original article

Determination of the races of *Plasmopara halstedii* (Farl.) Berl. & de Toni, the causal agent of sunflower downy mildew in Turkey and reactions of some commercial sunflower varieties against these races

Ayçiçeği mildiyösü etmeni *Plasmopara halstedii* (Farl.) Berl. & de Toni'nin Türkiye'deki ırklarının tespiti ve bazı ticari ayçiçeği çeşitlerinin bu ırklara karşı reaksiyonlarının belirlenmesi

Erçin OKSAL[a*], Salih MADEN[b]

[a]*Malatya Turgut Özal University, Faculty of Agriculture, Department of Plant Protection, Malatya, Turkey*
[b]*Ankara University, Faculty of Agriculture, Department of Plant Protection, Ankara, Turkey*

ARTICLE INFO

ABSTRACT

Downy mildew of sunflower caused by *Plasmopara halstedii*, is the most important disease of sunflower throughout the world including Turkey. Within the scope of the study, surveys were performed in Tekirdağ, Edirne, Kırklareli, Ankara, Bursa, Samsun, Tokat and Adana provinces where sunflower is widely grown in the period of 2009-2015. During the surveys, sixty-five *P. halstedii* isolates were obtained and purified. Using the race differentials set, nine races of the pathogen (100, 102, 110, 300, 500, 502, 510, 702 and 712) were determined. All races determined are first records for Turkey, whereas the races 102, 510 and 110 are the first record for the world. Almost 71% percent of the races were belonged to race 100. The reactions of 19 commercial sunflower cultivars against *P. halstedii* were determined by using isolates representing different races of downy mildew. None of the commercial sunflower cultivars showed resistance against all the races of *P. halstedii* at the same time. Among varieties tested LG5580 was found to be resistant to four races, whereas Sanay MR, Sanbro MR, LG540HO, and Sanbro varieties were found to be 3,3,2 and 1 races, respectively.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important source of vegetable oil and keeps gaining popularity because of its high oil percentage and quality, short growth duration and thermos-photo insensitiveness. It provides 46% of the total vegetable crude oil production in Turkey.

Most of the oilseed sunflower of Turkey is grown in Thrace and Marmara region (47.2%) and it is followed by Central Anatolia (29.2%), Black Sea (12%) and Mediterranean region (8.7%). About 2.000.000 tons of sunflower production is obtained from 795.215 ha cultivated area (Anonymous 2018). Sunflower oil consumption in Turkey is approximately 650.000 tons annually in which 400.000-450.000 tons provided by domestically.

More than 30 diseases of sunflowers have been documented worldwide (Gulya et al. 1991). Downy mildew is the most destructive disease affecting sunflower worldwide (Kolte 1985). The presence of sunflower downy mildew in Turkey was firstly reported in 1958 in Sakarya province (Karel 1958). In 2007 and 2008, a sunflower downy mildew outbreak occurred in Thrace and Marmara regions at four leaf period and crop losses up to 85% occurred (Göre 2009).

Plasmopara halstedii (Farl.) Berl. & de Toni is an obligate oomycete plant pathogen which remains viable in the soil as oospores for up to 10 years (Hall 1989, Nishimura 1926, Novotel'nova 1966, Sackston 1981). Under favourable conditions, oospores germinate to give zoosporangia. Zoospores formed in the zoosporangia cause primary infections of sunflower radicles, leading to systemic infection that cause most of the losses. The systemic infection of the plants results stunting, leaf chlorosis, horizontal head, poor seed set and severe yield reduction (Komjati 2010). Disease severity may considerably varies according to region, year and growing conditions. The disease is especially severe when high moisture and cool temperatures (14–16 °C) prevail at 2-4 leaf growth stages. The prevalence of sunflowers contaminated with *P. halstedii* in a field may be ranged from traces to near 50% or even up to 95% (Sackston 1981).

P. halstedii was originated from the North America and spread into Europe in the early 1940's. It remained pathologically uniform until the introduction of resistant sunflower cultivars. The physiological races (pathotypes) of *P. halstedii* display variation in the interaction with sunflower genotypes. The nomenclature of downy mildew pathotypes is based on an internationally accepted methodology using a set of sunflower differential lines with distinct resistance-susceptibility reactions (Gulya et al. 1998). The most detailed and up-to-date list of global distribution of *P. halstedii* pathotypes has been compiled by Gulya (2007) and Viranyi et al. (2015). In this accurate overview, he comprised as many as thirty-five pathotypes, an unbelievably high number considering the fact that in most sunflower producing countries from just a few to 12 well-distinguished virulence phenotypes exist (Viranyi 2008). The quantity and composition of pathogen races vary in different countries

and the determination of these is the main objective of the studies by leading phytopathologists (Kormany and Viranyi 1997, Masirevic 1992, Molinero-Ruiz et al. 2002, Penaud et al. 1997, Rozynek and Spring 2001, Shindrova 2005, 2010, Shirshikar 2005, Tourvieille de Labrouhe et al. 2000).

While the number of pathotypes increasing, new pathotype evaluation techniques are emerging. Sedlarova et al. (2016) determined two new races (race 705 and 715) from, from the single site in The Check Republic by evaluating the resistance/susceptibility by five-digit code.

In the study carried out by Shindrova (2010) in Bulgaria, the race composition of *P. halstedii* was found to be quite stable in the years 1988-2000 and with the introduction of tolerant or resistant cultivars, the number of the races increased up to five.

There has not been a study on race differentiation of sunflower downy mildew up to now in Turkey, although Viranyi et al. (2015) listed 9 races, most of them belonging over the seven hundreds, from 700 to 774. They did not quote any reference about those races, some of which maintained by regional Agricultural Research Institute of Thrace and May Seed company.

The composition of sunflower cultivars widely grown in Turkey has been changed a lot and newly bred hybrid varieties have been introduced and grown in various places of Turkey. Until 2019, no research related with races of *P. halstedii* had been carried out. The aim of this study is to determine the races of *P. halstedii* according to the three-digit nomenclature system and the reaction of some commercial sunflower cultivars against all the determined pathotypes of downy mildew in Turkey.

MATERIALS AND METHODS

Plasmopara halstedii populations

Sixty-five *P. halstedii* populations were collected from sunflower fields in eight provinces of Turkey; in eight different sunflower fields of Turkey; namely Edirne, Tekirdağ, Kırklareli, Bursa, Adana, Samsun, Tokat and Ankara provinces, during years 2009 and 2015. The zoosporangia of downy mildew were directly recovered from individual plants showing sporulation on their lower sporulation on lower surface of leaves.

Obtain monozoosporangial isolates

Inoculum was obtained from infected leaves (Figure 1). Each sporulated leaf was placed in a 50 ml Falcon conical tube containing 20 ml of NaCl solution (9 g NaCl + 1 liter distilled water) and shaken. A hundred microlitres of inoculum

suspension was spread Petri on the surface of water agar medium on 9 cm diameter disposable Petri dishes (15 g agar / liter distilled water). The zoosporangia were collected individually with a Pasteur pipette. The sunflower (*Helianthus annuus* line Ha-89, universal susceptible) leaves were rinsed with sterile water to reduce microbial infection. Leaf disks were cut from the first pair of sunflower leaves when they were 5 to 8 cm long. The disks were cut with a 5 mm diameter cork borer. The disks were placed in ELISA plates (Nunc-Immuno™ MicroWell™ 96 well solid plates, Germany) with the lower surface in contact with dilute salt solution (DS) that each well was filled with 300 µl DS, containing 10 µg/ml rifampicin (Machlis 1958). Then the inoculum prepared as mentioned above was placed on a leaf disk in a drop (20 µl) of distilled water under a stereo microscope (Leica, Switzerland). The plates were wrapped with Parafilm and incubated in a climate chamber (Panasonic, Japan) at 18 ºC for 16 h photoperiod. They were observed every day until sporulation. The disks which developed sporulation were individually placed in to an Eppendorf tube with 1 ml of distilled water. One pre-germinated seedlings (24-48 h, 24 ºC on moistened filter paper) of line HA-89 was also placed into the Eppendorf tube for 4 h. This seed was then transferred to a 9 cm diameter plastic pot containing perlite/sand mixture (2/3, v/v). The pots were maintained in a climate chamber at 24 ºC, 65-70% relative humidity (RH) in 16 h photoperiod (12000 lux) for incubation 10-14 days then inoculated sunflower seedlings were placed overnight in a dark climate chamber at 100% RH and 18 ºC (24-48 h), to initiate sporulation. The zoosporangia obtained from the infected seedlings were considered as mono-zoosporangial isolates. These isolates were multiplied on line Ha-89 seedlings using the method of Cohen and Sackston (1973).

Figure 1. An infected sunflower plant by *Plasmopara halstedii* having profuse sporulation used to obtain mono-zoosporangial isolates

Inoculum preparation

The surface of sunflower seeds were disinfected in 15% sodium hypochloride solution for 10-15 min, washed with tap water, placed on moistened filter paper and put in a dark climate chamber at 24 ºC. Seed inoculation method of *P. halstedii* described by Cohen and Sackston (1973) and modified by Molinero-Ruiz et al. (2002) was used for determination resistance/susceptibility. When the radicle was 2–5 mm long, they were immersed for 4 h at 18 ºC in an inoculum suspension 3×10^4 zoosporangia/ml containing 25 µg/ml riboflavin and 2 mg/ml glucose to increase the activity of zoosporangia. Then five seedlings were sown in each plastic pot (9 cm diameter) filled with perlite/sand mixture (2/3, v/v). In a climate chamber (24 ºC) in 16 h photoperiod, plants were grown for 10–12 days until first pair of true leaves (in size of mouse ears) developed. Then the plants and inside of the pots were moistened with distilled water to obtain relative humidity. Inoculated sunflower seedlings were placed in a dark climate chamber overnight to initiate sporulation on cotyledons and/or the first pair of true leaves of susceptible seedlings (Figure 2) (Molinero-Ruiz et al. 2002).

Figure 2. Initiate sporulation on cotyledons and/or the first pair of true leaves of susceptible seedlings

Race characterization

The method used in race characterization was the same as used at inoculum preparation stage (Cohen and Sackston 1973, Molinero-Ruiz et al. 2002). Sunflowers were scored as resistant (R) if no sporulation was seen on cotyledon and as susceptible (S) if sporulation was observed in cotyledons and the first pair of true leaves. The races of the *P. halstedii* populations were identified by using the triplet coding

system (Table 1) that records the R/S reactions of each nine lines of sunflower differential set by Gulya et al. 1998 (Table 2). There were five replications per each differential line (5 seedlings in each replication), and the entire experiment was repeated twice.

Table 1. Triplet coding system for defining sunflower downy mildew races (Gulya et al. 1998)

Differential Line	D-1 D-2 D-3	D-4 D-5 D-6	D-7 D-8 D-9	Triplet Code
Value if S.	1　2　4	1　2　4	1　2　4	
European race	S　R　R	S　R　R	S　R　R	100
	1 + 0 +　0 = 1	0 + 0 +　0= 0	0 + 0 +　0= 0	
Red River race	S　S　R	R　R　R	R　R　R	300
	1 + 2 + 0= 3	0 + 0 +　0= 0	0 + 0 +　0= 0	
Race xyz	S　S　S	S　R　S	R　S　R	752
	1 + 2 +　4= 7	1 + 0 + 4= 5	0 + 2 +　0= 2	

S = susceptible; R = resistant; Race "xyz" = hypothetical race

Table 2. Sunflower differential lines for downy mildew race identification

Designation	Original Name	Pedigree	Source of Resistance
D-1	Ha-89 (USDA)*	-	-
D-2	Rha-265 (USDA)	Peredovik/953-102	953-102 (Canada)
D-3	Rha-274 (USDA)	HA-119/HA-62	953-88 (Canada)
D-4	DM-2 (INRA)**	selection of PMI-3	Novinka (Russia)
D-5	PMI-17 (USDA)	PI 406022	? (Iran)
D-6	803-1(IFVC)***	*H. tuberosus*	*H. tuberosus*
D-7	HAR-4 (USDA)	Saenz-Pena 74-1-2	? (Argentina)
D-8	HAR-5 (INRA)	QHP-1 (INRA)	Guayacan INTA (Argentina)
D-9	HA-335 (USDA)	HA-89 x wild *H. annuus*	wild *H. annuus*

* INRA, 234 avenue du Brézet, 63039 Clermont-Fd cedex02, France

**USDA Northern Crop Science Laboratory, Fargo, ND 58105 USA

***IFVC, Maksima Gorkog 30, 21000 NoviSad, Yugoslavia

Reactions of some commercial sunflower cultivars against downy mildew races

The reaction of nineteen commercial sunflower cultivars (LG 540HO, LG 5580, LG 5550, LG 5650 CL, Sanbro MR, Sanay MR, Sanbro, Oleko, Alhaja, Transol, Dkf 2525, Bosfora, Dkf 3518, Biser Cl, Sirena, Armada Cl, Es Primus, Es Amira and Aitana) against *P. halstedii* was determined by using isolates representing each of nine races. The method used in race characterization was also used for inoculum preparation (Cohen and Sackston 1973, Molinero-Ruiz et al. 2002). Sunflowers were scored as mentioned above for race characterization. There were five replications per each cultivar (5 seedlings in each replication), and the entire experiment was repeated twice per each race.

RESULTS

Occurrence of sunflower downy mildew in Turkey

A total of 65 fields were found to be infested by downy mildew, out of the 296 sunflower fields inspected between the years of 2009 and 2015. The average incidence of affected fields was 25%, which we expected yield losses to occur (Figure 3). The survey area comprised four climatically different regions of Turkey; Thrace and Marmara, Black Sea, Central Anatolia and Mediterranean regions.

Figure 3. A sunflower field showing dwarfed sunflowers infected by *Plasmopara halstedii* near Edirne province

Races of Plasmopara halstedii in Turkey and their distribution

Analysis of 65 monozoosporangial isolates of *P. halstedii* obtained from the same number of fields, yielded 9 races determined by the reactions of three sets of differential varieties. Distribution of the races is given in Table 3.

The most widespread race was "race 100" which was obtained from all the provinces and 70.7% of the infested fields. The second most widespread race was "race 500" obtained 10 (15%) samples from four of the provinces. Race 300 was found in 4 of the samples while races 102, 110, 502, 510, 702 and 712 were obtained from only one sample each.

The highest number of races were obtained from Kırklareli, followed by Tekirdağ and Edirne provinces, which all are located in Eastern Thrace region and comprise the highest amount of sunflower cultivated area in Turkey.

Reactions of some commercial sunflower cultivars against Plasmopara halstedii

Fifteen of the sunflower cultivars did not show any resistance against the all of *P. halstedii* determined in Turkey (Table 4). Five cultivars; LG 5580, LG 5400 HO, Sanay MR, Sanbro and Sanbro MR, were resistant to race 100. There were not any resistance against the races 102, 110, 510, 702 and 710. LG 5580 showed resistant reaction against four races.

Table 3. Distribution of the races of *Plasmopara halstedii* in Turkey

Provinces	Plasmopara halstedii Races								
	100	**102**	**110**	**300**	**500**	**502**	**510**	**702**	**712**
Adana	3	-	-	-	-	-	-	-	-
Ankara	2	-	-	-	-	-	-	-	-
Bursa	7	1	-	-	1	-	-	-	-
Edirne	3	-	1	2	3	-	-	-	-
Kırklareli	14	-	-	1	5	1	1	1	1
Samsun	2	-	-	-	-	-	-	-	-
Tekirdağ	11	-	-	1	1	-	-	-	-
Tokat	3	-	-	-	-	-	-	-	-
Total	45	1	1	4	10	1	1	1	1

Table 4. Reactions of twenty sunflower cultivars against nine races of *Plasmopara halstedii*

Cultivars tested	Plasmopara halstedii Races								
	100	**102**	**110**	**300**	**500**	**502**	**510**	**702**	**712**
LG 5580	R	S	S	R	R	R	S	S	S
SANAY MR	R	S	S	R	R	S	S	S	S
SANBRO MR	R	S	S	R	R	S	S	S	S
LG 5400 HO	R	S	S	R	S	S	S	S	S
SANBRO	R	S	S	S	S	S	S	S	S
LG550	S	S	S	S	S	S	S	S	S
LG 5650 CL	S	S	S	S	S	S	S	S	S
OLEKO	S	S	S	S	S	S	S	S	S
ALHAJA	S	S	S	S	S	S	S	S	S
TRANSOL	S	S	S	S	S	S	S	S	S
DKF 2525	S	S	S	S	S	S	S	S	S
BOSFORA	S	S	S	S	S	S	S	S	S
DKF 3518	S	S	S	S	S	S	S	S	S
BISER CL	S	S	S	S	S	S	S	S	S
SIRENA	S	S	S	S	S	S	S	S	S
ES PRIMUS	S	S	S	S	S	S	S	S	S
ES AMIRA	S	S	S	S	S	S	S	S	S
AITANA	S	S	S	S	S	S	S	S	S
ARMADA CL	S	S	S	S	S	S	S	S	S

(S = susceptible; R = resistant)

DISCUSSION

A total of 296 sunflower fields were inspected for *Plasmopara halstedii* incidence between 2009 and 2015 (Figure 3) and about 25% of them were found be infested. The rate of the infestation is expected to increase since *P. halstedii* survive in the soil a long time and a lot of new cultivars have been introducing which could bring new races although the rate of seed transmission is very low.

Although there is a review by Viranyi et al. (2015) giving the number of the races of *P. halstedii* in Turkey, no citation, related with them, has been mentioned in this paper. Races of *P. halstedii* were firstly reported with this study. By the past 6 years occurrence or introduction of new races could be possible since many new sunflower cultivars have been

either imported or bred. It is highly probable that the races mentioned by Viranyi et al. (2015) exist in Turkey.

Races of *P. halstedii* were determined by using nine differential lines. About 70% of the population of *P. halstedii* were belong to race 100 (45 out of 65), followed by race 500 and 300. Races 100, 102, 110, 300, 500, 502, 510, 702 and 712. Races 102, 110 and 510 were recorded for the first time in the world. Recently, race determination with nine differentials has been found insufficient for some races and a new set comprising 15 lines have been adopted. Mostly new races over 7 digits have increased especially after the introduction of new resistant cultivars (Molinero-Ruiz 2018, Sedlarova et al. 2016, Shindrova 2010).

There is only one study on the reactions of some sunflower

cultivars and lines against *P. halstedii* races in Turkey. They evaluated the resistance of different cultivars and lines except Sanbro MR, which was found resistant races 703 and 710. This cultivar was also found resistant to three other races (Race 100, 300 and 500) but not to races 702. Çiftçigil et al. (2014) prepared a spore mixture with sunflower downy mildew isolates collected from Thrace and Marmara region and applied the mixture to 22 sunflower genotypes. The result of the study was used to develop new resistant genotypes against the disease. In a similar study, Evci et al. (2011) collected the leaves of sunflower infected with downy mildew from Thrace region and prepared a spore mixture. They artificially inoculated the mixture to some sunflower lines and they observed the reactions of these plant against the disease. They detected a difference in resistance in some lines as a result of the study. Unlike this study, downy mildew spores were used as bulk, not mono zoosporangial in both studies.

Seed treatment is a very effective way to control sunflower downy mildew provided that no fungicide resistance occurs, which has not been documented so far. Seed treatment is also useful to control other soil borne diseases. Growing resistant varieties is a good option for organic production and race determination is required since reactions of the cultivars varies according to the races but this should be done with frequent intervals.

ACKNOWLEDGEMENT

ÖZET

Plasmopara halstedii (Farl.) Berl. & de Toni'nin neden olduğu Ayçiçeği mildiyösü Türkiye'de dahil olmak üzere dünyada ayçiçeğinin en önemli hastalığıdır. Çalışma kapsamında 2009-2015 yılında Türkiye'nin yoğun ayçiçeği tarımı yapılan Tekirdağ, Edirne, Kırklareli, Ankara, Bursa, Samsun, Tokat ve Adana illerinde sürveyler yapılmıştır. Sürveyler sırasında altmışbeş *P. halstedii* izolatı elde edilmiş ve saflaştırılmıştır. Irk farklılık seti kullanılarak patojenin dokuz farklı ırkı (100, 102, 110, 300, 500, 502, 510, 702, 712) tespit edilmiştir. Tespit edilen ırkların hepsi Türkiye için ilk kayıt niteliği taşırken ırk 102, 510 ve 110 Dünya için ilk kayıt niteliğindedir. Irkların %71'i ırk 100'e aittir. Ayçiçeği mildiyösünün farklı ırkları kullanılarak 19 adet ticari ayçiçeği çeşidinin de ırklara karşı reaksiyonları belirlenmiştir. Ticari ayçiçeği çeşitlerinin hiçbirisi *P. halstedii*'nin ırklarının tamamına karşı dayanıklılık göstermemiştir. Sırasıyla Sanbro 1, LG540HO 2, Sanbro MR 3 ve Sanay MR 3 ırka dayanıklılık gösterirken, LG5580 4 ırka dayanıklı olarak bulunmuştur.

Anahtar kelimeler: *Plasmopara halstedii,* ırk, çeşitler, ayçiçeği

REFERENCES

Anonymous 2018. Ayçiçeği raporu. http://www.zmo.org.tr/ (Erişim tarihi: 20.10.2019).

Cohen Y., Sackston W.E., 1973. Factors affecting infection of sunflower by *Plasmopara halstedii*. Canadian Journal of Botany, 51, 15–22.

Çiftçigil T.H., Evci G., Pekcan V., Yılmaz M.İ., Kaya Y., 2014. Determination of resistance of some sunflower genotypes against downy mildew utilizing from artificial inoculation. Joint International Congress 14th Mediterranean Phytopathological Union, 25-29 August 2014, İstanbul, Turkey, 150 s.

Evci G., Akın K., Kaya Y., Pekcan V., Yılmaz M., 2011. The determination of downy mildew (*Plasmopara halstedii* (Farl.) Berl. & De Toni.) resistance of some sunflower lines in Trakya region. Anadolu, 21 (1), 36-43.

Göre M.E., 2009. Epidemic outbreaks of downy mildew caused by *Plasmopara halstedii* on sunflower in Thrace, part of the Marmara region of Turkey. Plant Pathology, 58, 396.

Gulya T.J., 2007. Distribution of *Plasmopara halstedii* races from sunflower around the world. Advances in Downy Mildew Research, Vol 3, Palacky University and JOLA Publishers, 121–134 pp.

Gulya T.J., Sackston W.E., Virányi F., Masirevi S., Rashid K.Y., 1991. New races of the sunflower downy mildew pathogen (*Plasmopara halstedii*) in Europe and North and South America. Journal of Phytopathology, 132 (4), 303-311.

Gulya T.J., Tourvieille de Labrouhe D., Masirevic S., Penaud A., Rashid K., Viranyi F., 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). In: Gulya T., Vear F., (Eds.). Third Sunflower Downy Mildew Symposium, Fargo, USA, ISA, 130-136.

Hall G., 1989. Unusual or interesting records of plant pathogenic Oomycetes. Plant Pathology, 38 (4), 604-611.

Karel G., 1958. A preliminary list of plant diseases in Turkey. Ayyıldız Matbaası, Ankara, 44 pp.

Kolte S.J., 1985. Diseases of annual edible oilseed crops. 3, Sunflower, Safflower & Niger. CRS press, Inc., Boca Raton, USA, 154 pp.

Komjáti H., 2010. Phenotipic and molecular genetic characterisation of sunflower infecting *Plasmopara* populations. PhD Thesis, Szent István University, Gödöllő, 16 pp.

Kormány A., Virányi F., 1997. Studies on the virulence and agressiveness of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. In Proceedings 49th International Symposium on Crop Protection, Mededelingen Faculteit Landbouwkundige Universiteit, Gent, 62/3b, 911–915.

Machlis L., 1958. Evidence for a sexual hormone in Allomyces. Physiologia Plantarum, 11, 181-192.

Molinero-Ruiz M.L., Domínguez J., Melero-Vara J.M., 2002. Races of isolates of *Plasmopara halstedii* from Spain and studies on their virulence. Plant Disease, 86 (7), 736-740.

Molinero-Ruiz L., 2018. Recent advances on the characterization and control of sunflower soilborne pathogens under climate change conditions. OCL (oilseeds and fats, crops and limits), 26 (2), 9 pp.

Maširević S., 1992. Rase prouzrokovača plamenjače *Plasmopara halstedii* kod nas i u svetu (in Serbian). Periodicals of Institute of Field and Vegetable Crops Novi Sad, 20, 405-409.

Nishimura M., 1926. Studies in *Plasmopara halstedii*. Journal of the College of Agriculture, Hokkaido Imperial University. 11, 185-210.

Novoteľnova N.S., 1966. Taxonomy and biology of the sunflower mildew, downy mildew of sunflower. USSR, Nauka, Moscow, 150 pp.

Penaud A., Delos M., Lafon S., Walser P., De Guenin M.C., Tourvieille J., Molinero V., Tourvieille D., 1997. Evolution du mildiou du tournesol en France 1, 407-412 Compte-rendu de la 5ème Conférence Internationale sur les Maladies des Plantes, Tours, France.

Rozynek B., Spring O., 2001. Leaf disc inoculation, a fast and precise test for the screening of metalaxyl tolerance in sunflower downy mildew. Journal of Phytopathology, 149, 309-312.

Sackston W.E., 1981. Downy mildew of sunflower. In D.M. Spencer (Ed.), The downy mildews, London, Academic, 545–575.

Sedlářová M., Pospíchalová R., Drábková Trojanová Z., Bartušek T., Slobodianová L., Lebeda A., 2016. First report of *Plasmopara halstedii* new races 705 and 715 on sunflower from the Czech Republic – short communication. Plant Protection Science, 52.

Shindrova P., 2005. New nomenclature of downy mildew races in sunflower (*Plasmopara halstedii* Farl. Berlese et de toni) in Bulgaria (race composition during 2000-2003). Helia, 28 (42), 57-64.

Shindrova P., 2010. Investigation on the race composition of downy mildew (*Plasmopara halstedii* Farl. Berlese et de Tony) in Bulgaria during 2007-2008. Helia, 33, 52, 19-24.

Shirshikar S.P., 2005. Present status of sunflower downy mildew disease in India. Helia, 28 (43), 153-158.

Tourvieille L.D., Gulya T.J., Masirevic S., Penaud A., Rashid K.Y., Viranyi F., 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). In: Proceedings of the 15th International Sunflower Conference, Toulouse, 2, (I), 61–66 pp.

Viranyi F., 2008. Research progress in sunflower diseases and their management. Proceedings, 17th International Sunflower Conference, Córdoba, Spain.

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.