PALACKÝ UNIVERSITY IN OLOMOUC

FACULTY OF SCIENCE

DEPARTMENT OF BOTANY

Zuzana Drábková Trojanová

PATHOGENIC VARIABILITY AND STUDY OF INTERACTIONS WITHIN *HELIANTHUS* SPP. - *PLASMOPARA HALSTEDII* PATHOSYSTEM

Ph.D. Thesis

Ph.D. Program P1527 V Biology, 15-07-9 Botany

Supervisor: Doc. RNDr. Michaela Sedlářová, Ph.D.

Olomouc 2017

The work was supported by the Ministry of Agriculture of the Czech Republic (project no. QH71254), Ministry of Education, Youth and Sports (MSM 6198959215), European Social Fund (projects BioLink CZ.1.07/2.4.00/17.0007, InterDoc OPVK CZ.1.07/2.4.00/17.0008, FytoChem OPVK CZ.1.07/2.200/28.0171) and internal grants of Palacký University in Olomouc (IGA_UP PrF_2012_01, PrF_2013_003, PrF_2014_001, PrF_2015_001, PrF_2016_001, PrF_2017_001).

Declaration

Hereby I declare, that I have elaborated my Ph.D. thesis independently using literary sources and materials included in the list of references.

In Olomouc 15. 5. 2017

.....

Zuzana Drábková Trojanová

ACKNOWLEDGEMENTS

I wish to express my deep and sincere gratitude to:

My supervisor Doc. RNDr. Michaela Sedlářová, Ph.D. for offering me a chance and conditions to participate in this research at the Department of Botany, her professional, kind and patient supervision, guidance and support from the very beginning up to this moment. Furthermore I am heartily thankful to her for thorough reading of this thesis and all my publications and for her valuable and constructive advices. Last but not least I would like to thank for a life-changing experience.

Prof. Ing. Aleš Lebeda, DrSc. for his supervision, invaluable advices, critical comments, reading of all publications and mediating contacts with external specialists.

Prof. Dr. Otmar Spring for supervision and guidance during internships in Hohenheim University, Germany, his valuable advices and extending my horizons.

RNDr. Miloslav Kitner, Ph.D and his colleagues from the laboratory of molecular markers for guidance, valuable advices and help with experiments.

Students Romana Pospíchalová, Tomáš Bartůšek, Karel Stojaspal, Tereza Doudová and Lucie Slobodianová for collaboration on *Plasmopara halstedii* research.

Reinhard Zipper, Wolfgang Grasse, Javier Goméz and other colleagues from Hohenheim University for sharing experience, valuable advices and helping hand.

Drahomíra Vondráková and Věra Zoubková for their technical assistance and taking care of experimental plants.

Finally, I would like to thank to all my former and current colleagues, family and to my best friend and husband Pavel Drábek for helping me to get through the difficult moments, their patience, support, motivation and caring they have provided.

In Horažďovice, February 28th 2017 Zuzana Drábková Trojanová

BIBLIOGRAPHIC IDENTIFICATION

Author's first name and surname: Mgr. Zuzana Drábková Trojanová

Title: Pathogenic variability and study of interactions within *Helianthus* spp. - *Plasmopara halstedii* pathosystem

Type of thesis: Ph.D. Thesis

Workplace: Department of Botany, Faculty of Science, Palacký University in Olomouc,

Šlechtitelů 27, 783 71 Olomouc-Holice

Supervisor: Doc. RNDr. Michaela Sedlářová, Ph.D. (michaela.sedlarova@upol.cz)

The year of presentation: 2017

Abstract:

This Ph.D. thesis focuses on biotrophic parasitic oomycete *Plasmopara halstedii*, the cause of sunflower downy mildew, a quarantine disease spread globally. Following a critical review of methodology used in *P. halstedii* research, it brings the first overview of pathogenic variability in *P. halstedii* populations on cultivated sunflower in the Czech Republic. Since 2007, when detailed research was initiated, a limited but repeated incidence of sunflower downy mildew was recorded. Besides widely distributed races 700 and 710, also recently frequent races 704 and 714 were found, together with the first ever records of races 705 and 715. Data, obtained during last decade, are crucial for sunflower breeders and growers and fill in the white spot in the knowledge about *P. halstedii* variability in sunflower-producing countries in Europe.

Keywords: differential set, *Helianthus annuus* L., *Plasmopara halstedii*, quarantine disease, sunflower downy mildew, virulence formula

Number of pages: 92

Number of appendices: 1

Language: English

BIBLIOGRAFICKÁ IDENTIFIKACE

Jméno a příjmení autora: Mgr. Zuzana Drábková Trojanová

Téma: Patogenní variabilita a studium interakcí v patosystému *Helianthus* spp. - *Plasmopara halstedii*

Druh práce: Doktorská disertační

Pracoviště: Katedra botaniky, Přírodovědecká fakulta, Univerzita Palackého v Olomouci, Šlechtitelů 27, 783 71 Olomouc-Holice

Školitel: Doc. RNDr. Michaela Sedlářová, Ph.D. (michaela.sedlarova@upol.cz)

Rok obhajoby práce: 2017

Abstrakt:

Předkládaná disertační práce se zabývá biotrofní parazitickou oomycetou *P. halstedii*, která je původcem karanténní choroby zvané plísňovitost slunečnice. Po úvodním kritickém přehledu metod, používaných při studiu *P. halstedii*, přináší tato práce první informace o patogenní variabilitě populací *P. halstedii* na pěstovaných slunečnicích v České republice. Kromě celosvětově rozšířených ras 700 a 710 byly zaznamenány také v poslední době časté rasy 704 a 714 a především poprvé na světě vůbec odhaleny rasy 705 a 715. Informace získané během posledního desetiletí jsou klíčové zejména pro šlechtitele a pěstitele slunečnic ve střední Evropě, vyplňují prázdné místo na mapě poznání a rozšiřují vědomosti o *P. halstedii* v Evropě.

Keywords: difrenciační soubor, *Helianthus annuus* L., karanténní choroba, *Plasmopara halstedii*, plísňovitost slunečnice, virulence

Počet stran: 92

Počet příloh: 1

Jazyk: anglicky

CONTENTS

1. INTRODUCTION

Biotrophic oomycete *Plasmopara halstedii* (Farlow) Berlese & de Toni (1888) is a causal agent of sunflower downy mildew, one of the major diseases affecting cultivated sunflowers worldwide. Sunflower is the fourth most intensively grown oil crop following oil palm, soybean and rapeseed (Gascuel et al., 2015). In the Czech Republic sunflower represents the third major oil crop after rapeseed and poppy (Ritschelová et al., 2016). Sunflower is cultivated for food, animal feed production and marginally for alternative biofuel industry (Sackston, 1981). Sunflower downy mildew causing damping off the seedlings, plant dwarfing and flower sterility, may result in economic loses up to 100% of seed production. Massive crop devastation occurs especially when factors such as susceptible sunflower cultivars, soil contaminated by *P. halstedii* oospores and suitable climatic conditions meet. As practices of integrated pest management are being deployed, the yield losses due to downy mildew are recently estimated to 3,5 % of the global commercial sunflower seed production (Gascuel et al., 2015).

Pathogenic variability of sunflower downy mildew has been known for more than 50 years but sunflower cultivars bearing dominant resistance (*Pl*) genes able to control the disease have not been described before 1980´s (Vear, 2016). Long lasting selection pressure caused by employing *P. halstedii* resistant sunflower hybrids finally resulted in evolution of plethora of new virulent forms and physiological races overcoming *Pl* genes (Ahmed et al., 2012) which continues ever since (Gulya, 2007; Virányi et al., 2015; Sedlářová et al., 2016). Disease management based on fungicide application appeared to be only temporary solution, as resistance to metalaxyl emerged in the pathogen populations (Albourie et al., 1998; García-Ruiz et al., 2012).

P. halstedii is apparently capable of rapid genetic evolution (Ahmed et al., 2012; Virányi et al., 2015) and its homothallic nature allows the pathogen to colonize new localities even by a single zoospore (Spring, 2000). Both factors designate *P. halstedii* as dangerous pathogen, and it is no wonder that sunflower downy mildew has been declared a quarantine pathogen in the European Union since 1992 (Delmotte et al., 2008). Distribution and pathogenic variability of *P. halstedii* has been continuously monitored in U.S. and Europe since 1970´s (Gulya, 2007; Virányi et al., 2015), but only scattered information on *P. halstedii* were available from the former Czechoslovakia and the Czech Republic, respectively. To fill this gap a project focused on monitoring of disease occurrence and variability of *P. halstedii* populations was initiated by prof. A. Lebeda and doc. M. Sedlářová at our department in 2007. Literature overview was compiled and methodology of pathogen sampling, cultivation and variability testing was reviewed and optimized (Trojanová et al., 2017).

This thesis brings the first summary of *P. halstedii* research in the Czech Republic. It contains proposal for optimal *P. halstedii* cultivation, deposition and virulence testing, detailed study of its geographic distribution and harmfulness in CR as well as results obtained during eight years of monitoring of *P. halstedii* physiological races on cultivated sunflowers.

2. AIMS OF THE PH.D. THESIS

This thesis compiles up-to-date knowledge about *Helianthus annuus* - *Plasmopara halstedii* pathosystem with own original data and accessible historical records to provide complex insight into *P. halstedii* problematics in the Czech Republic set in the context of the Central European and global populations of the pathogen. It aims to:

- optimize methods of *P. halstedii* sampling (collection, isolation), handling (inoculation, cultivation, maintenance, conservation) and determination of virulence phenotype on host differential set;
- monitor geographic distribution, incidence and harmfulness of *P. halstedii* in CR;
- determine races of collected *P. halstedii* isolates based on virulence phenotype of sunflower differential set.

3. LITERATURE OVERVIEW

3.1 Taxonomy and phylogeny of *Plasmopara halstedii*

Plasmopara halstedii (Farlow) Berlese & de Toni (1888) is an oomycete obligate biotrophic parasite causing downy mildew of sunflower and other more than hundred taxa from Asteraceae family. Oomycetes had been traditionally studied by mycologists due to their morphological and ecological similarities with fungi, but molecular and biochemical data showed relation of these organisms to heterokont algae which led to their re-classification into kingdom Chromista (syn. Stramenopila, Straminipili) (Dick, 2001). Recent phylogenetic studies suggest that oomycetes belong to clade of eukaryotic Biconts called the "SAR" supergroup, including Stramenopila, Alveolata and Rhizaria (Adl et al., 2012). Taxonomic position of *P. halstedii* (Tab. 1) underwent several modifications since the first description of the organism by Farlow in 1883, which was reviewed e.g. by Virányi et Spring (2011).

Tab. 1. Recent scientific classification of *Plasmopara halstedii*

Taxonomic rank	
Domain	Eukarya
(unranked)	SAR
Superphyllum	Heterokonta
Phylum	Oomycota
Class	Oomycetes
Order	Peronosporales
Family	Peronosporaceae
Genus	*Plasmopara*
Species	*Plasmopara halstedii*

In precise determination of a downy mildew morphological, biological and molecular characteristics together with host range must be taken into account. Originally *P. halstedii* was described by Farlow (1883) as *Peronospora halstedii*. In 1886 the genus *Plasmopara* was separated from original genus *Peronospora* by Schrötter based on differences in germination, i.e. by formation of zoospores instead of germ tubes. *P. halstedii* has been studied and morphologically described in details since 1920´s (Nishimura, 1922), however variability in size and shape of *P. halstedii* sporangiophores and sporangia was reported if formed on different organs of a host plant (Zahka et Viranyi, 1991).

Traditional determination of downy mildew based on light microscopy of morphological features, such as sporangia and sporangiophores, became inappropriate following appearance of molecular methods and description of new species (Thines, 2007). Despite of mathematic tools and image analysis able to discriminate tiny details, the downy mildews in general provide low morphological complexity, i.e. limited number of characters

suitable for routine classification based on light microscopy (Sedlář et al., 2009). Downy mildews determination based on (conidio)sporangia and (conidio)sporangiophores (Waterhouse, 1973) or haustoria (Voglmayr et Constantinescu, 2008) usually distinguishes organisms reliably only at a genus level. Thines (2007) proposed SEM-visualized characters to be better applicable for classification and phylogeny purposes.

Host range of *P. halstedii* comprises of many species from Asteraceae family and thus defines a polyphyletic species complex (Voglmayr et Constantinescu, 2008). Historical suggestion made by Farlow (1883) to separate *Peronospora halstedii* (= *Plasmopara halstedii*) on Asteriodeae from *P. ganglioniformis* (= *Bremia lactucae*) on Cichorioidae, i.e. according to host subfamilies, was proven insufficient (Virányi et Spring, 2011). Recent phylogenetic studies led to *Plasmopara* species revision and reclassification resulting in separation of newly introduced genera such as *Novotelnova, Protobremia* or *Plasmoverna* (Voglmayr et Constantinescu 2008, Virányi et Spring, 2011). Spring et Haas (2002) showed that composition of fatty acids was unique in *P. halstedii*. However, fatty acid profiling has never been broadly accepted as a taxonomic character. Newly, specific protein profiles represent a promising tool available to distinguish individual downy mildew taxa (Chalupová et al., 2012). Nevertheless, the major part of current knowledge has been gained thanks to the development of molecular methods.

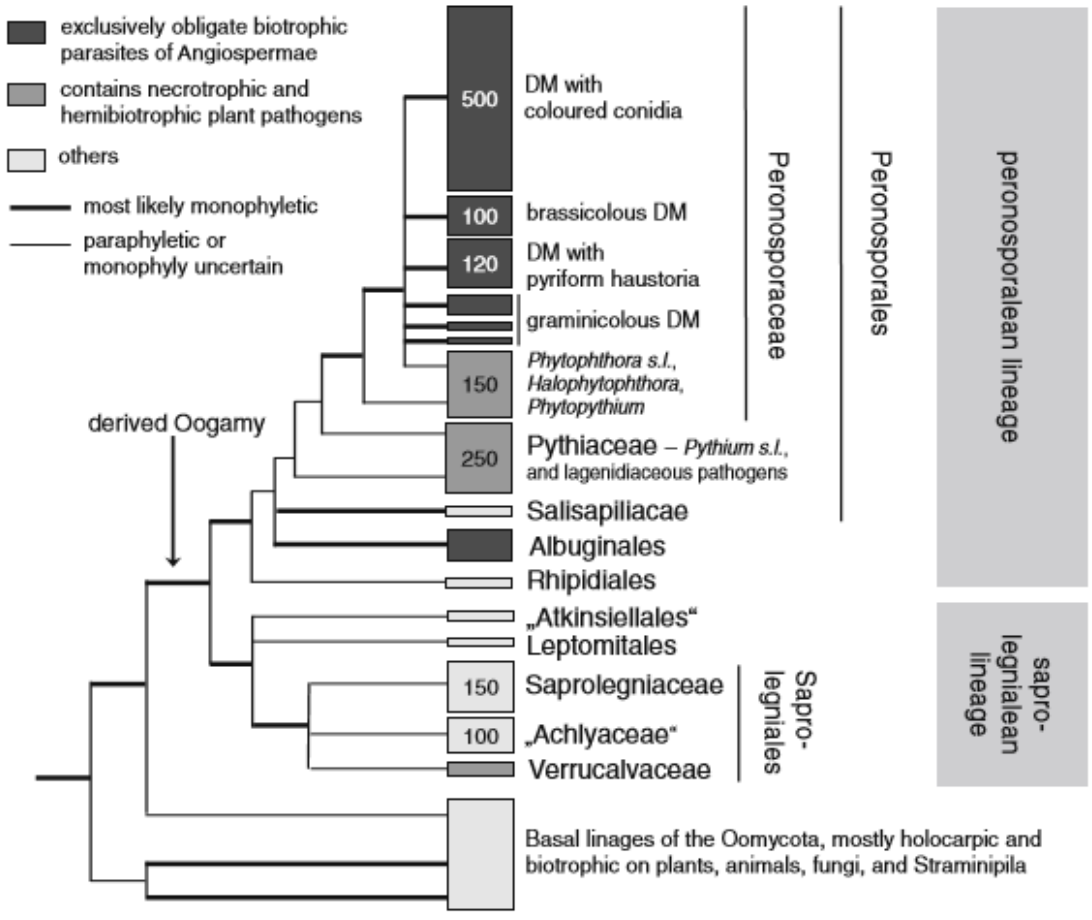
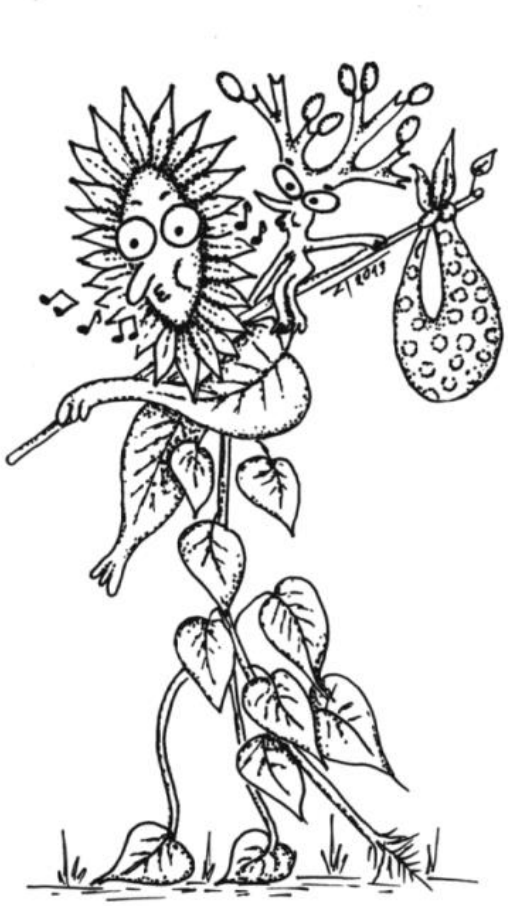


Fig. 1. Overview of oomycete phylogeny with respect to downy mildews (adopted from Thines, 2014).

Actual data suggest that *P. halstedii* belongs to a group of exclusively biotrophic downy mildews with pyriform haustoria, parasites on Angiospermae (Thines, 2014; Fig 1.). Biotrophic pathogens causing downy mildews can be divided into at least four morphological and ecological subgroups based on sequences of LSU, COX 2, β-tubulin and NADH 1 (Gascuel et al., 2015). Three subgroups are highly probable to be monophyletic: downy mildews with coloured sporangia (genus *Peronospora* and *Pseudoperonospora*), downy mildews with vesicular to pyriform haustoria containing genus *Plasmopara* (and *Basidiophora*, *Bremia*, *Benua*, *Paraperonospora*, *Plasmoverna* and *Protobremia*), and downy mildews on Brassicaceae (*Hyaloperonospora* and *Perofascia*). Monophyletic relationships in the fourth group, including grass parasites *Viennotia oplesmeni*, *Graminivora graminicola* and *Sclerospora graminicola*, were not clearly proven (Göker et al., 2007; Thines, 2014).

Recently, the genus *Plasmopara* was proven as polyphyletic and split into six entities: core *Plasmopara*, *Novotelnova* (*Pl. savulescui*), *Plasmoverna* (*Pl. pygmaea* s.l.), *Poakatesthia* (*Pl. penniseti*), *Protobremia* (*Pl. sphaerosperma*), and *Viennotia* (*Pl. oplismeni*) (Voglmayr et Constantinescu, 2008). Nowadays, the genus *Plasmopara* harbours at least 109 species and together with the genus *Peronospora* it represents globally the most abundant oomycetes (Lebeda et al., 2006).



3.2 Up-to-date state of knowledge about *P. halstedii*

Plasmopara halstedii is native to North America where it was first identified by B. D. Halsted on *Eupatorium purpureum* in 1876 (Nishimura, 1922). The first report of *P. halstedii* on cultivated sunflower comes from USA in 1920´s (Young et Morris, 1927). In Europe it was recorded for the first time from Russia in 1960´s, probably introduced *via* infected sunflower seed (Ioos et al., 2007). Nowadays, *P. halstedii* is present in 52 countries around the globe including North (3), Central (1) and South (5) America, Europe (23), Asia (13) and Africa (7) (CABI, 2017; Fig. 2). *P. halstedii* has been absent from Australia and Oceania; downy mildew found in that region on *Arctotis* and *Arctotheca* belong to closely related species *Plasmopara majewskii* (Constantinescu et Thines, 2010). *P. halstedii* has been considered a quarantine disease under regulation in the European Union since 1992 (Gascuel et al., 2015) and in the Czech Republic since 2002 (Spurný, 2005).

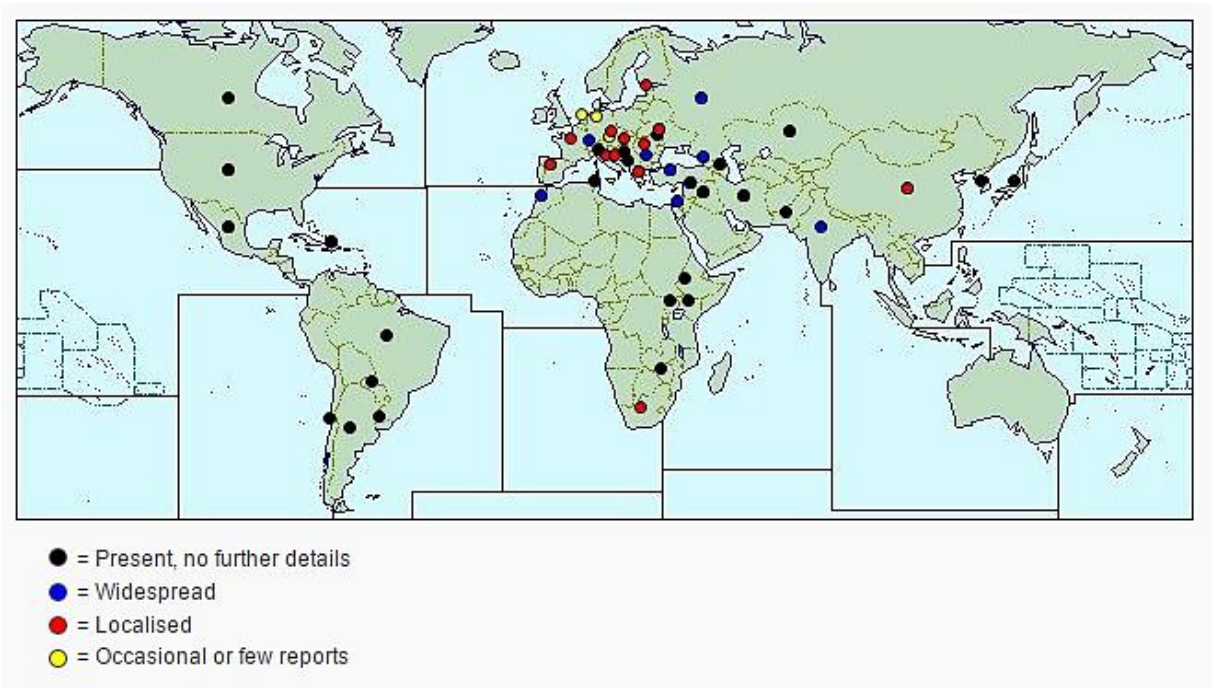


Fig. 2. Global distribution of *P. halstedii* (adopted from CABI, 2017).

Genome of *P. halstedii* has been completely sequenced and compared with genomes of other taxa belonging to both obligate biotrophic and saprotrophic oomycetes (Sharma et al., 2015). Genome of *P. halstedii*, similarly to other obligate biotrophic downy mildews, is richer in AT than that of pathogens with saprotrophic lifestyle. The genome of *P. halstedii* is known for a low rate of heterozygosity which is consistent with homothallic nature of the pathogen. Selfing through homothallism can be an adaptation for ensuring sexual reproduction and production of overwintering oospores even though host plants are distributed sparsely (Sharma et al., 2015). Homothallism after rare events of parasexual recombination probably helps to emergence of isolates resistant to fungicides or virulent on a variety of sunflower cultivars containing R-genes (Rozynek et Spring, 2000).

During plant infection oomycetes secrete an arsenal of effector proteins that target host extracellular (apoplastic effectors) or intracellular metabolic processes (cytoplasmic effectors) and thus enable its colonisation (Kamoun, 2006). Oomycete effectors are the major virulence determinants recognized by host R proteins during so called gene-for-gene interaction (Kamoun, 2007). During plant-pathogen coevolution the composition of effectors in oomycetes undergoes a selective pressure resulting in rapid diversification of gene sequences (Schornack et al., 2009). Thus effector gene polymorphism can be exploited for molecular differentiation of races (Gascuel et al., 2016). *P. halstedii* genome contains sequences coding oomycete effectors as well, so far 77 CRN-like and 247 RxRL-like motifs were detected during genome analysis (Sharma et al., 2015) and subsequent secretome analysis revealed 27 CRN and 27 RXLR effectors (Gascuel et al., 2016). The percentage of polymorphism in *P. halstedii* effector genes is twice as high as for non-effector genes showing resistance evolution. Therefore, effectors detected by Gascuel et al. (2016) were used for designing KASP (Competitive Allele Specific PCR) markers that were successfully used for discrimination of some *P. halstedii* pathotypes.

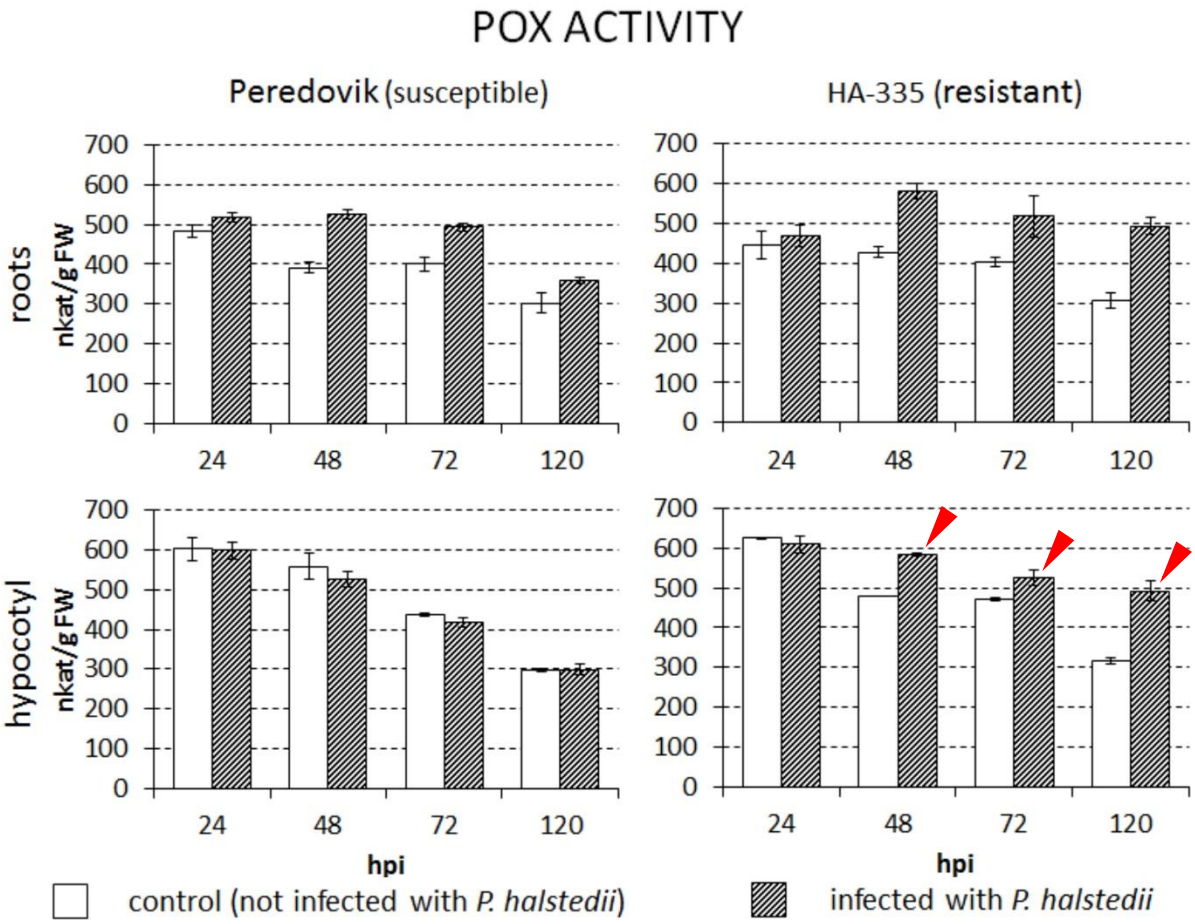


Fig. 3. Peroxidase (POX) activity in roots and hypocotyl of sunflower during compatible (susceptible host) and incompatible (resistant host) interaction with *P. halstedii*. Increased POX activity was observed in roots of both susceptible and resistant hosts, but in hypocotyl it was increased only in resistant sunflower genotype (arrows), indicating enzyme involvement in the host defense reaction (Drábková Trojanová, unpublished data).

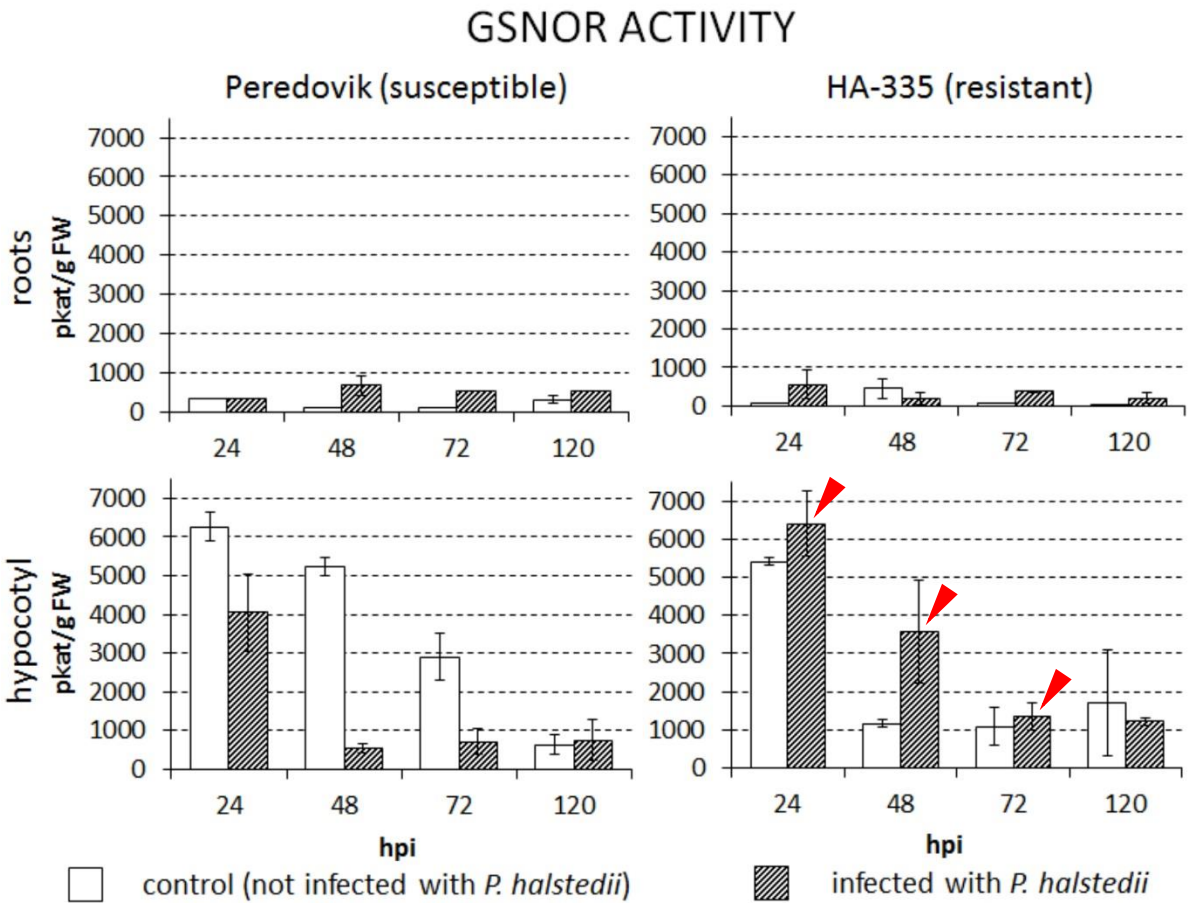


Fig. 4. Changes in activity of *S*-nitrosoglutathione reductase (GSNOR) in roots and hypocotyl of sunflower during compatible (susceptible host) and incompatible (resistant host) interaction with *P. halstedii*. Increased GSNOR activity in resistant host hypocotyl (arrows) indicates nitrosative stress induced by the pathogen (Drábková Trojanová, unpublished data).

Interaction of sunflower with *P. halstedii* results in production of a broad spectrum of metabolites, PR proteins and signal molecules originating from both partners. *P. halstedii* itself seems to possess the capacity to produce phytohormones from classes of brassinolides and cytokinins (Sharma et al., 2015), as well as antagonists of phytohormones, e.g. IAA oxidase resulting in dwarfism of systemically infected sunflowers (Benz et Spring, 1995). Early after inoculation *P. halstedii* induces oxidative and nitrosative stress in the host plant tissues (Chaki et al., 2009) which stimulates activity of both peroxidase and *S*-nitrosoglutathione reductase in hypocotyl of resistant genotype (Fig. 3 and 4). During incompatible interaction it corresponds with hypersensitive reaction taking place also in hypocotyls which stops *P. halstedii* colonisation (Radwan et al., 2005). Activity of extracellular invertase, a pathogenesis related protein which catalyses hydrolysis of sucrose to fructose and glucose utilized by the pathogen (Roitsch et al., 2003), is increased in compatible sunflower - *P. halstedii* interaction (Trojanová, 2010).

3.3 Biology, epidemiology and host range of *P. halstedii*

Sunflower downy mildew is a homothallic (Spring, 2001) obligate biotrophic parasite which requires living host tissues to accomplish its life cycle (Gascuel et al., 2015). The life cycle is complex (Fig. 5), including both sexual and asexual reproduction (Nishimura, 1922). *P. halstedii* genetic variability can be increased by rare events of outcrossing or parasexual recombination (Spring et Zipper, 2006). Host infection is mediated by motile biflagellate zoospores which germinate from sporangia in humid and cold conditions (ca 10-15 °C, Virányi et Spring, 2011), dispersion of the pathogen within host population is mediated *via* asexually formed sporangia. Survival of the pathogen to the next season is assured by durable oospores formed by sexual reproduction (Gascuel et al., 2015).

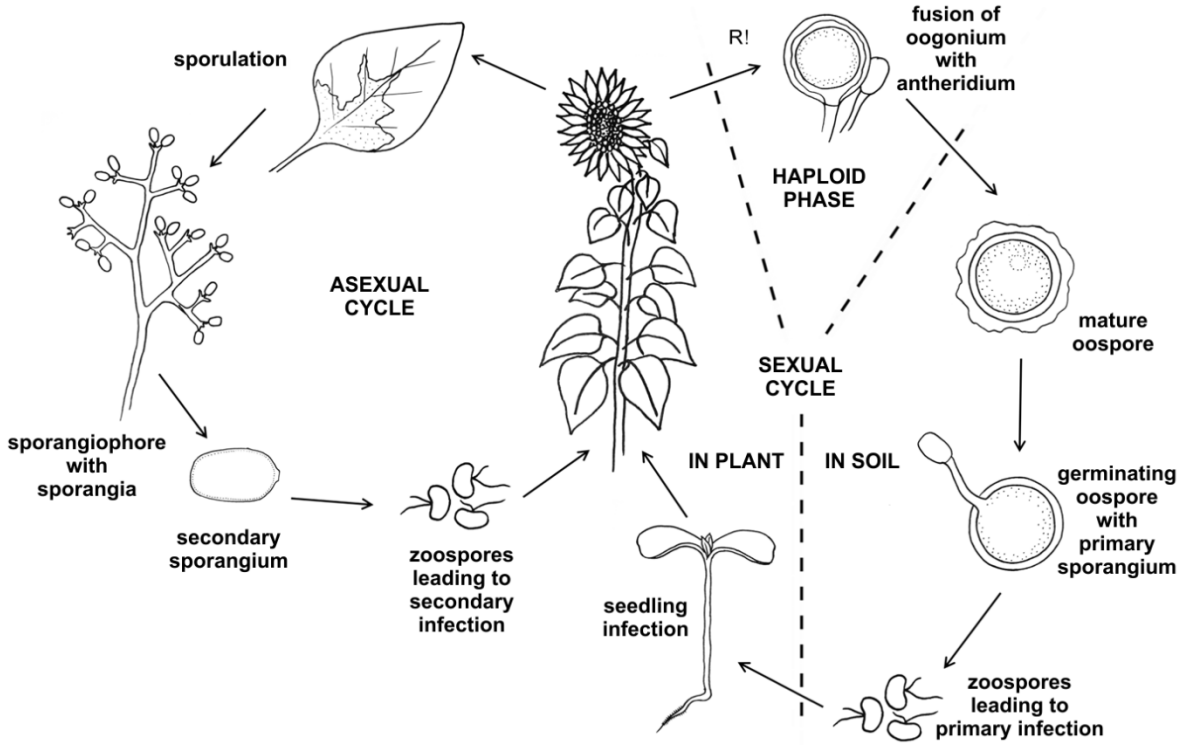


Fig. 5. Life cycle of *Plasmopara halstedii* (compiled according to Spring, 2001)

In spring, soil-born primary infection of host seedlings is caused by overwintering oospores (Gascuel et al., 2015). Zoospores are released from a single primary sporangium emerging from oospore, travel in water film towards seedling roots or hypocotyl, encystate and penetrate host rhizo- or epidermis (Virányi, 1988). In compatible interaction, coenocytic hyaline mycelium grows intercellularly within tissues, haustoria penetrate the host cells to provide nutrition and pathogen gradually colonizes the plant causing symptoms of systemic infection (Sedlářová et al., 2010). After successful host colonization, hyaline laterally branched sporangiophores grow out of stomata or penetrate host epidermis to produce several generations of secondary sporangia. These are released in huge amounts and disseminated by wind which may lead to disease epidemics in host population. Mitotically formed zoospores germinate from sporangia deposited on the surface of plants and cause

secondary infections. At the end of growing season sexual reproduction takes place, when male antheridium and female oogonium originating from the same mycelium fuse and durable oospores are produced (Spring, 2001).

Sunflowers infected by *P. halstedii* show symptoms with intensity depending on type of infection and plant developmental stage. Soil-born primary infections lead to most frequent systemic infection affecting the whole host plant causing seedling damping-off, dwarfism, chlorosis and formation of deformed and straight upward towering capitulum. Weak primary infections may occasionally turn into cotyledon limited type of latent infection producing plants without clear symptoms except for sporulation on hypocotyl and cotyledons (Tourvieille de Labrouhe et al., 2000a). Wind-born secondary infections can lead into frequent local infections forming restricted chlorotic leaf lesions with pathogen sporulation which can remain local (Heller et al., 1997) or evolve into systemic infection towards inflorescence (Spring, 2009). Late secondary infection may lead into latent infections with mycelium restricted only to sunflower receptacle and achenes, leaving the host plant symptomless (Spring, 2001).

P. halstedii is predominantly soil-borne and seed-borne pathogen (CABI, 2017) but wind-borne infections are significant for epidemiology as well (Spring, 2001). The most susceptible stage of host development is between germination and emergence of seedlings (Meliala et al., 2000) when the whole plantlet surface is in contact with the soil-borne inoculum in the form of zoospores. Since zoospores require free water to reach the host, disease risk rises with the appearance of precipitation shortly after sowing (Tourvieille de Labrouhe et al., 2008b). Air temperature has clear effect on disease incidence as well; the most favourable are mean air temperatures between 10 and 15 °C during the 5 days after sowing (Virányi et Spring, 2011)

Each systemic infection (primary, evolved from local infection or latent infection of receptacle) that reaches up to inflorescence may produce seeds containing *P. halstedii* mycelium or oospores (Spring, 2001). Seeds from systematically infected sunflowers produce systematically infected offspring in 2% of cases (Döken, 1989) however these from latent infected plants in higher rates (Spring, 2001). Contaminated seeds are thus able to transport the pathogen on long distances, e.g. from one continent to another (Virányi, 2002). Each type of compatible interaction in theory enables the pathogen to complete its sexual reproduction. Therefore even one infected plant that survives till the end of the growing season represents a potential source of highly durable inoculum which might establish the pathogen on new locality. Oospores are capable of survival in the soil for as long as 8-10 years. Therefore the sunflower downy mildew is extremely difficult to eradicate once it has been established at a locality (CABI, 2017). Disease transmission on local level is mediated mainly by soil particles with oospores, e.g. within a field during tillage (Virányi et Spring, 2011), and by wind-distributed sporangia (Spring, 2001). However, the extent of secondary infections is highly dependent on favourable (wet and cool) weather conditions (Virányi et Spring, 2011). Moreover, airborne inoculum facilitates encountering and hybridisation of different *P. halstedii* strains coexisting in one area (both different pathotypes and inocula

from non-sunflower hosts) and thus generating intraspecific variability (Ahmed et al., 2012).

Tab 2. One of the first lists of *P. halstedii* host range *sensu lato* in North America published by Wilson in 1908.

Genus	Species	Genus	Species
Ageratina	*altissima*	*Helianthus*	*maximiliani*
Ambrosia	*artemisiifolia*		*occidentalis*
Artemisia	*ludoviciana*		*strumosus*
Bidens	*cernua*		*tuberosus*
	comosa	*Iva*	*xanthiifolia*
	connata	*Madia*	*sativa*
	frondosa	*Rudbeckia*	*fulgida*
	laevis		*laciniata*
Centaurea	sp.		*triloba*
Erechtites	*hieraciifolia*	*Silphium*	*laciniatum*
Erigeron	*annuus*		*perfoliatum*
Eupatorium	*purpureum*		*terebinthinaceum*
Gnaphalium	*purpureum*		*trifoliatum*
	spathulatum	*Solidago*	*canadensis*
Helianthus	*annuus*		*riddellii*
	divaricatus	*Vernonia*	*baldwinii*
	× *doronicoides*		*noveboracensis*
	grosseserratus	*Verbesina*	*encelioides*
	hirsutus	*Xanthium*	*strumarium*
	× *laetiflorus*		

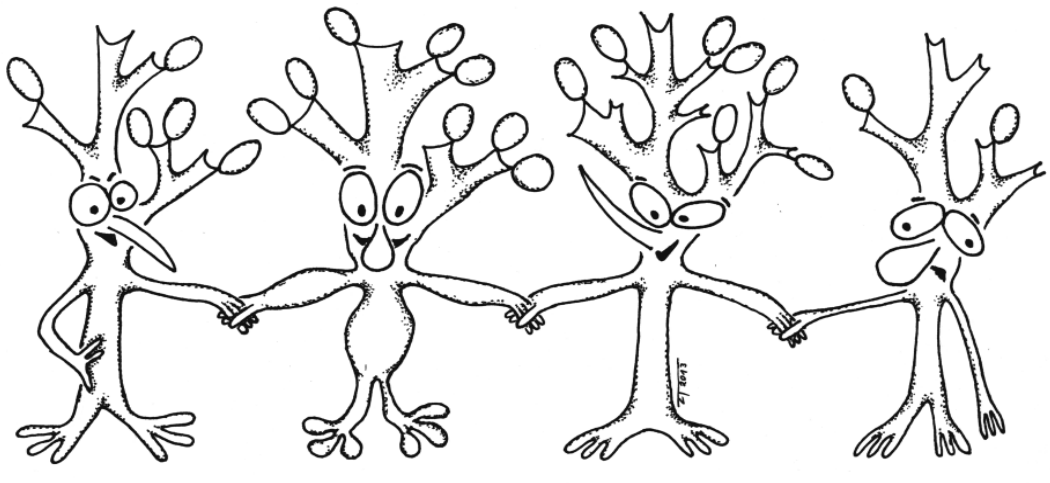
Host range of *P. halstedii* can be understood in *sensu lato* or *sensu stricto* species concept but always it harbours host plants from Asteraceae family. *Sensu lato* host range represents group of more than 80 species of the Asteraceae family (Gulya et al., 1997) and this attitude prevails in historical literature, e.g. Wilson (1908) listed 40 species in 17 genera as hosts of *P. halstedii* in the North America (Tab. 2). Host range *sensu stricto* was based on inoculation tests with *P. halstedii* inoculum from cultivated sunflower on more than 70 potential hosts and cross inoculation tests with inocula from wild *P. halstedii* hosts on cultivated sunflower (for review see Virányi et Spring, 2011; Tab. 3.) and later supported by molecular data (Choi et al., 2009a). However, some wild Asteraceae hosts such as *Ageratum houstonianum* and *Ceratopsis lanceolata* were determined using only morphometric features without any molecular biology backup (Mattos et al., 2006, Choi et al., 2009b). On the contrary *Plasmopara* on *Xanthium strumarium* was based on SSR and rDNA sequences reclassified to *P. angustiterminalis* (Komjáti et al., 2007). It implies that host range of *P. halstedii* is not settled yet and revision of potential hosts will require cross inoculation tests and molecular analyses.

Tab. 3. Proposed narrow (*sensu stricto*) host range of *P. halstedii*.

Sunflower hosts confirmed by inoculation tests	Wild hosts confirmed with cross-inoculation tests	Wild hosts unconfirmed by any inoculation test
H. agrophyllus	*Ambrosia artemisiifolia* (Walcz et al., 2000)	*Ageratum houstonianum* (Mattos et al., 2006)
H. annuus	*Artemisia vulgaris* (Walcz et al., 2000)	*Ceratopsis lanceolata* (Choi et al., 2009b)
H. deblils	*H.× laetiflorus* (Spring et al, 2003)	
H. divaricatus	*Iva xanthiifolia* (Gulya, 2002)	
H. grosserratus	*Rudbeckia fulgida* (Rivera et al., 2016)	
H. lenticularis	*Xanthium strumarium* * (Komjáti et al., 2007)	
H. petiolaris **		
H.× multiflorus		
H. tuberosus		

* Downy mildew was reclassified according to molecular genetic investigation as *P. angustiterminalis* Novot.

** Walcz et al., 2000



3.4 Variability in populations of *Plasmopara halstedii*

Pathogenic variability in populations of downy mildews infecting economically important crops (such as *Bremia lactucae* on lettuce, *Plasmopara viticola* on grapevine, *Pseudoperonospora cubensis* on cucurbits) has been intensively studied since the 2nd half of 20th century. Physiological specialization in *Plasmopara halstedii* was proposed in between races with different geographic origin in early 1970´s (Orellana, 1970; Zimmer, 1974). Up to date more than 44 races, capable to overcome some resistance genes (*Pl*) incorporated into sunflower, have been described (Virányi et al., 2015; Sedlářová et al., 2016). Physiological races of *P. halstedii* denoted by three-digit codes differ in their specific reaction on a set of nine sunflower genotypes (Fig. 6) (Tourvieille de Labrouhe et al., 2000b); newly recognized virulence patterns are composed from five-digit codes based on reaction of fifteen differentials (Tourvieille de Labrouhe et al., 2012; Gascuel et al., 2015). Differential set consisting of *Helianthus annuus* lines bearing unique combinations of resistance genes has been under continuous development since 1990´s (reviewed in Trojanová et al., 2017). Despite intensive testing during last quarter century only recently molecular markers for rather precise determination of *P. halstedii* races have been designed (Gascuel et al., 2016).

Fig. 6. Phenotype of sunflower differentials reaction to infection by a *P. halstedii* isolate. Susceptible differential lines with sporulation of *P. halstedii* are marked by arrows.

Intraspecific pathogenic variability in *P. halstedii* is known since 1970´s when Zimmer (1974) described sunflower lines resistant to *P. halstedii* isolates originating from Europe but susceptible to those from North America. These two clusters of *P. halstedii*

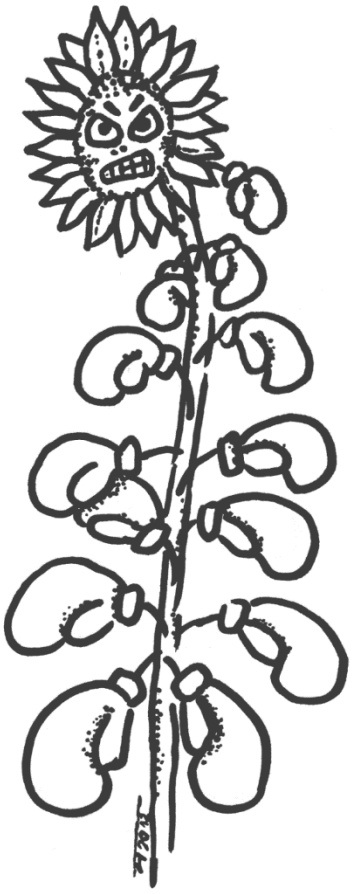
isolates were later described as European race (race 1 or newly race 100) and Red River Valley race (race 2, or race 300) (Sackston et al., 1990). In 1980 a new race of *P. halstedii* able to overcome *Pl2* gene, until then effective against Red River Valley race, was reported from South Dakota, USA (Carson, 1981) which was later described as race 3 (700) (Gulya et al., 1982). In 1985 race 4 (730) was identified in Minnesota (Gulya et Urs, 1985) and race 5 (770) was described 1988 in North America (Ljubich et al., 1988). Several other races were detected later which initiated changes in methodology of tests and race terminology (Gulya et al., 1991a). Availability of relevant data also relates to a period which the pathogenic variability stood in the centre of attention in individual countries. In France, for example, the number of recorded *P. halstedii* pathotypes increased from the only one in 1987 to fourteen in 2011 (Ahmed et al., 2012). Globally, at least 36 races were documented in 2006 (Gulya, 2007) which number increased to current at least 44 races (Trojanová et al., 2017). Moreover a shift in occurrence of individual races has been recorded in *P. halstedii* populations during the last decades (Tab 4) (Virányi et al., 2015).

Tab. 4. Distribution of *P. halstedii* races in Europe before (X) and after (Y) the year 2007 (adopted from Virányi et al., 2015).

Vir. Code	BG	CZ	F	D	H	I	RO	RUS	SRB	E	TR
100	X		X	X,Y	X,Y	X	X,Y	X,Y	X,Y	X,Y	
300	X,Y		X	X,Y	X	X	X,Y	X,Y		X,Y	X
304			X,Y								
307			X,Y								
310				X,Y			Y	X,Y		X,Y	
314			X,Y								
320				Y							
330	X,Y			X,Y	X,Y		X,Y	X,Y		X,Y	X
334			X,Y								
700	X,Y	Y	X		X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X
703			X,Y			X,Y				X,Y	X
704		Y	X,Y		Y	Y					
707			X,Y								
710		Y	X,Y	X,Y	X,Y		Y	X,Y		X,Y	X
713											X
714		Y	X,Y		Y						
717			X,Y								
721	Y										
730		Y	X,Y	X,Y	X,Y		X,Y	X,Y	X,Y	X,Y	X
731	Y										
750				Y							
770		Y	Y	X,Y						Y	X
773											X
774			Y								
Total	4+5	0+6	14+13	7+9	6+7	4+3	5+7	7+7	3+3	8+9	9+0

Notes: Explanation: X means before 2007, Y means after 2007.
BG = Bulgaria, CZ = Czech Republic, *F* = France, *D* = Germany, *H* = Hungary, *I* = Italy, RO = Romania, RU = Russia, SRB = Serbia, *E* = Spain, TR = Turkey.

Tests based on host differential phenotyping are time and material demanding (Gascuel et al., 2016) thus molecular tools have been tested over the time. Isozyme analysis of *P. halstedii* isolates revealed differences in between isolates originating from different hosts, but it was not capable of detecting races (Komjáti et al., 2008). Several attempts to use molecular approaches, such as RAPD (Random amplified polymorphic DNA) (Borovkova et al., 1992; Guchetl et al., 2012), RFLP (restriction fragment length polymorphism) (Bovkorov et al., 1993), TARP (target region amplification polymorphism) (Chen et al., 2005), ITS (sequence of internal transcribed spacer region of nuclear ribosomal DNA) (Spring et al., 2006) or SSRs (microsatellite markers) (Slobodianová, 2016), were not successful in discrimination of *P. halstedii* races recognized by a phenotypic response of sunflower differential set. Attention of molecular biologists focused also on polymorphic markers related to host-pathogen interaction such as EST (expressed sequence tags) markers (Giresse et al., 2007) and finally Gascuel et al. (2016) succeeded in discriminating some (but not all) multi-isolate clusters belonging to the same physiologic race using 22 KASP (Competitive Allele Specific PCR) markers designed on polymorphic effector genes involved in the host-pathogen recognition. Hopefully, even more specific molecular markers will be designed in the future to facilitate fast routine race determination.



3.5 Sunflower downy mildew management

Breeding of resistant pedigree, i.e. employment of major resistance genes (*Pl*) and factors has been the major strategy used for *P. halstedii* control in sunflower monocultures. Apart from the major genes which effectivity can be overcome by a pathogenic shift in populations of the downy mildew also quantitative trait loci in sunflower genome are intensively studied to provide field resistance and thus decrease the degree of disease intensity and yield losses.

Great effort has been made to "improve" the crop by introgression of major resistance (R) genes originating from both cultivated and wild *Helianthus* species (Hu et al., 2010) such as annuals *H. annus, H. argophyllus and H. praecox* or perennials *H. grosseserratus, H. maximiliani, H. tuberosus*, etc. (Seiler, 1992). So called *Pl* genes provide complete, but race specific resistance (Tab. 5) (Vear et al., 2007) according to a gene-for-gene concept (Flor, 1955). Long lasting study of *Pl* genes conferring resistance to newly appearing *P. halstedii* races has already resulted in discovery of no less than 23 *Pl* genes (Liu et al., 2012) and still continues.

Tab. 5. Efficiency of *Pl* genes against *P. halstedii* races studied in France (adopted from Vear, 2004). R = resistant, s = susceptible.

Groups [a]	100	300	304 (2000)	304 (2002)	307	314	330 (USA)	330 (ESP)	700	703	704	710	714	Genes
	R	s	s	s	s	s	s	s	s	s	s	s	s	*Pl*1 (Rha 266, Rha 265)
	R	R	R	R	R	R	R	R	s	s	s	s	s	*Pl*2 (Rha 274)
	R	R	R	s	R	s	R	R	s	s	s	s	s	*Pl*2 (PSC8)
1	s	s	s	s	s	s	R	R	R	R	s	R	s	*Pl*7 (YVQ)
	R	R	s	s	s	s	R	R	R	R	s	R	s	*Pl*6 (Ha 335, YDQ)
	R	R	s	s	s	s	R	R	R	R	s	R	s	*Pl*7 (Ha 337, YEQ)
	R	R	R	R	s	R	R	R	R	R	s	R	s	*Pl*7 (83 RM)
13	R	R	R	R	R	R	R	R	R	R	R	R	R	*Pl*Arg (Rha 419)
	R	R	R	R	R	R	s	R	R	R	R	R	R	*Pl*5 (XRQ, YSQ)
	R	R	R	R	R	R	s	s	R	R	R	R	R	*Pl*? (PM 17)
6	R	R	R	R	R	R	R	R	R	R	R	R	R	*Pl*8 (Rha 340)
	R	R	R	R	R	R	R	R	R	R	s	R	s	*Pl*8
	R	R	R	R	R	R	R	R	R	R	R	R	R	*Pl*? (803-1)
	R	R	R	R	R	R	R	R	R	R	R	R	R	*Pl*? (QPR1)
	R	R	R	R	s	R	R	s	s	s	s	s	s	*Pl*4 (XA)
	R	R	R	s	R	s	R	R	s	s	s	s	s	*Pl*4 (XK)
?	R	R	R	R	s	R	R	R	R	s	R	R	R	*Pl*? (QHP1)
	R	R	R	R	R	s	s	s	R	R	R	s	s	*Pl*? (PMI3)
	R	s	R	R	R	R	R	R	R	s	R	R	s	*Pl*? (RHA 428)

[a] Groups of Gentzbittel et al. (1999) map

On the contrary, quantitative resistance provides more durable, however partial resistance that may be sufficient to avoid crop yield lost and may decrease the selection pressure on *P. halstedii* populations driven by major *Pl* genes (Vear et al., 2007). Four-year

study of resistance independent on major genes comprehensive hundreds of sunflower lines revealed that at least 50 inbred lines already possess significant level of quantitative resistance (Tourvielle de Labrouhe et al., 2008a) and that it should be possible to combine it with R genes in modern sunflower cultivars (Vincourt et al., 2012).

Practices of integrated plant protection combine growing of resistant sunflower cultivars with fungicide application. Chemical control against sunflower downy mildew includes fungicides based on metalaxyl and later approved metalaxyl-M (mefenoxam, R-metalaxyl) (CABI, 2017). Protective preparations containing both compounds are approved in the European Union (PPDB, 2017a; PPDB, 2017b). In the Czech Republic no fungicide for sunflower downy mildew control based on metalaxyl is registered (Povolný et Hampl, 2015), only those based on metalaxyl-M (eAGRI, 2017). Both compounds may be used to prevent soil-born infection *via* seed treatment (CABI, 2017) or used in foliar sprays to control air-borne disease (Turner, 2015).

Metalaxyl (formula $C_{15}H_{21}NO_4$ IUPAC name methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate; synonym 2-[(2,6-dimethylphenyl)-(2-methoxy-1-oxoethyl) amino] propanoic acid methyl ester; trade names: Ridomil, Subdue, Apron, Proturf) and its active enantiomer metalaxyl-M (= mefenoxam), are systemic fungicides known to affect RNA in oomycetes. The mode of action is based on lethal inhibition of proteosynthesis due to interference in esp. ribosomal RNA synthesis (Turner, 2015). Metalaxyl, the first of the phenylamide fungicide class was introduced to market in 1977 (Müller at Gisi, 2012) to control both air-borne and soil-borne diseases caused by oomycetes. The fungicide was originally marketed esp. for use against *P. infestans* but crop was devastated by a potato blight epidemic in Ireland in 1980 as resistance developed. Soon after the fungicide suffered severe resistance problems also in populations of *Pseudoperonospora cubensis*, *Peronospora tabacina*, and *Plasmopara viticola* (Gisi et Cohen, 1996). The first report of metalaxyl tolerant *P. halstedii* isolates in Europe comes from Hungary in 1980´s (Oros et Virányi 1984), while in North America the first resistant isolates appeared in 1995 (Gulya et al., 1999). Luckily, metalaxyl resistant *P. halstedii* isolates in the Czech Republic have not been recorded (Bartůšek, 2013). Metalaxyl-M was introduced two decades after metalaxyl (Nunninger et al., 1996) due to increasing number of resistant isolates in controlled oomycetes and to reduce the amount of chemicals dispersed in environment (Müller et Gisi, 2012) because the pure enantiomer should be twice efficient than original metalaxyl (Turner, 2015). However, Molinero-Ruiz et al. (2005) already recorded metalaxyl-M tolerant *P. halstedii* isolates in Spain. Resistance to phenylamides in general is considered to be monogenic, such as in the case of *Bremia lactucae* (Crute et Harrison, 1988). Therefore metalaxyl/metalaxyl-M tolerant isolates may rise by a single mutation, which represents the weak point and future risk for intensive usage of these fungicides which put selection pressure on the pathogen´s population. Therefore multipart fungicides such as combination of metalaxyl and mancozeb showing synergic effect and higher efficiency against *P. cubensis* (Samoucha et Cohen, 1984) or those with alternative active substances such as strobilurins (Sudisha et al., 2010) were tested.

Biological control may offer an eco-friendly alternative for disease control. Intensively studied phenomenon of induced plant resistance (Hammerschmidt, 1999) represents supportive method to traditional chemical disease control and resistance breeding. Biological control agents in *H. annuus* - *P. halstedii* pathosystem show promising results. Induced sunflower resistance resulting in reduced downy mildew disease symptoms and pathogen growth inhibition can be achieved by treatment with chemical activators such as BTH (benzo(1,2,3)-hiadiazole-7-carbothioic acid S-methyl ester) (Bán et al., 2004), BABA (DL-β-amino butyric acid) or INA (2,6-dichloroisonicotinic acid) (Körősi et al., 2009). Induced systemic resistance lowering the disease intensity was also detected after treating sunflower seeds with essential oil from *Bupleurum gibraltarium* (Fernandez-Ocaña et al., 2004) or chitosan (Nandeeshkumar et al., 2008a). Similar effect induced colonization of sunflowers roots by plant growth promoting fungi (PGPF) (Nagaraju et al., 2012; Nandeeshkumar et al., 2008b). Though biological control agents are not as effective as fungicide treatment, they fulfil requirements of sustainable agriculture and should not be neglected in the integrated pest control.

3.6 *Plasmopara halstedii virus*

In 1990 virus like particles were detected in *Plasmopara halstedii*, for the first time reported in race 300 causing abnormal sunflower downy mildew symptoms (twisted host leaves, limited sporulation) in North America (Gulya et al., 1990). Transmission electron microscopy proved presence of isomeric virions in pathogen infection structures at all stages of ontogeny, but never in cytoplasm of invaded plant cells, i.e. outside of the pathogen thallus (Gulya et al., 1992). Presence of virions was later observed in various isolates of *P. halstedii* regardless their origin, pathotype or reaction to fungicides (Heller-Dohmen et al., 2008). Recent extensive PCR-based screening disclosed *Plasmopara halstedii virus* (*PhV*) in ca 90% of samples, originating from both herbaria and fresh collections, and confirmed its global distribution (Grasse et Spring, 2015).

Plasmopara halstedii virus (*PhV*) represents ss(+)RNA virus which infects exclusively *P. halstedii* (Grasse et Spring, 2015) and causes its hypovirulence on host plants (Grasse et al., 2013). Isomeric *PhV* virions observed within different *P. halstedii* isolates have been identical, non-enveloped, sized 37 nm in diameter (Fig. 7) and often forming crystalline-like aggregations in *P. halstedii* cytoplasm (Heller-Dohmen et al., 2008). Virus RNA is divided into two segments coding RNA dependent RNA-polymerase (RdRp, RNA strand 1, ORF 2745 nucleotides) and coat protein (CP, RNA strand 2, ORF 1128 nucleotides) (Grasse et Spring, 2015) with molecular mass 36 kDa (Heller-Dohmen et at., 2011). *PhV* shows extremely low genetic variability (Grasse et Spring, 2015).

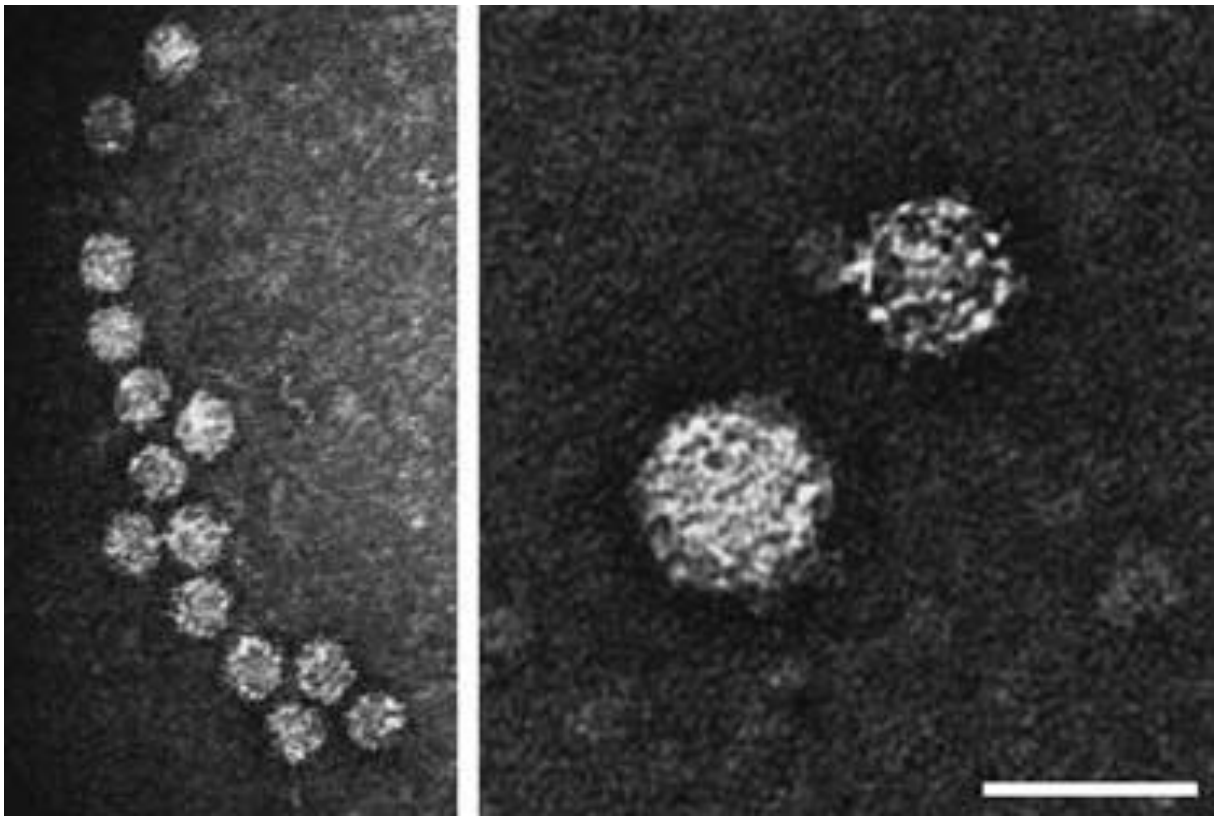


Fig. 7. *PhV* virions in TEM (adopted from Heller-Dohmen et al., 2008).

Only several oomycete species are known to harbour viruses or virus like particles (for review see Heller-Dohmen et al., 2008) and only two of them were studied in details, i.e. *Sclerophthora macrospora* Virus A (*SmV-A*, Yokoi et al., 2003) and *Sclerophthora macrospora* Virus B (*SmV-B*, Yokoi et al., 1999). Comparison of RNA sequences revealed that *SmV-A*, isolated from *S. macrospora* that causes downy mildew on a vast number of cereal crops including rice (*Oryza sativa* L.), represents virus the most similar to *PhV* (Heller-Dohmen et al., 2011). Since *PhV* sequence of RNA-dependent RNA polymerase (RdRp) shows similarities with viruses from the Nodaviridae family and shares coat protein (CP) sequence with Tombusviridae family (Fig. 8) (Heller-Dohmen et al., 2011), the taxonomic position of the *PhV* is ambiguous. Therefore Grasse et Spring (2017) proposed placing of *PhV*, together with *SmV-A* and Tombunodavirus UC1, in a new independent group between families Nodaviridae and Tombusviridae.

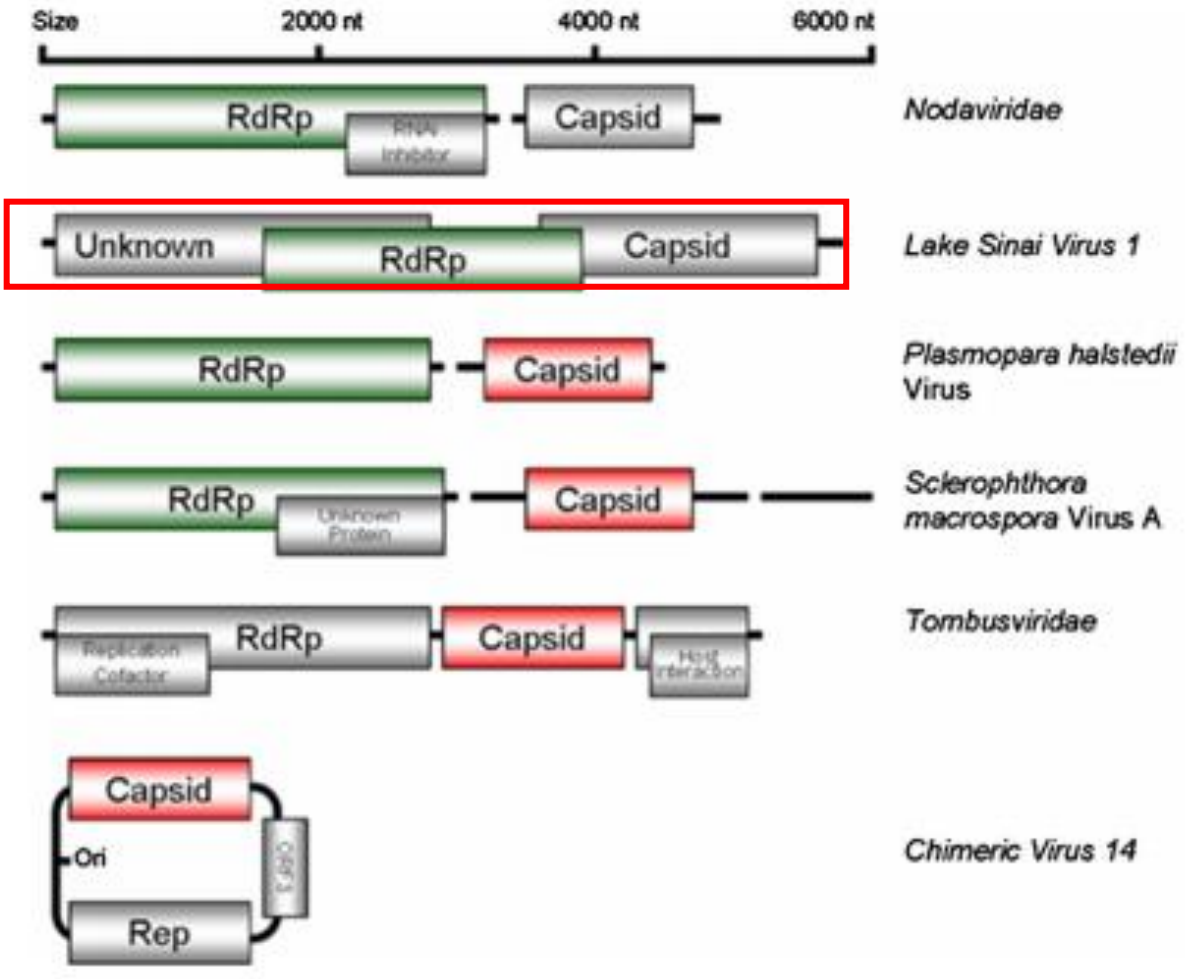


Fig 8. Genomic organization and proposed taxonomic position of *Plasmopara halstedii virus*. Green bars indicate RdRp similarities while red bars indicate CP similarities (adopted from Grasse et Spring, 2017).

4. RESULTS

4.1. Methodology

4.1.1. Trojanová, Z., Sedlářová, M., Gulya T.J., Lebeda A. (2017): Methodology of virulence screening and race characterization of *Plasmopara halstedii*, and resistance evaluation in sunflower – a review. Plant Pathology 66, 171–185.

Plant Pathology (2017) **66**, 171185 Doi: 10.1111/ppa.12593

REVIEW

Methodology of virulence screening and race characterization of *Plasmopara halstedii*, and resistance evaluation in sunower a review

Z. Trojanovâ[a], M. Sedlâřovâ[a], T. J. Gulya[bc] and A. Lebeda[a]*

[a]*Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelü 27, 783 71 Olomouc, Czech Republic;* [b]*Sunower and Plant Biology Research Unit, USDA-ARS, Fargo, ND, 58102; and* [c]*Department of Plant Pathology, North Dakota State University, Fargo, ND, 58102, USA*

Sunower downy mildew is a disease of high global economic impact as well as a causal agent that is extremely difcult to eradicate. During the past decades, several approaches for the determination of *Plasmopara halstedii* (Ph) races have been used worldwide and are discussed in this review. Procedures of isolation, cultivation and maintenance of Ph isolates, as well as the screening of sunower for resistance, are also critically reviewed. The predominant, globally used resistance screening protocol is a whole seedling immersion inoculation. Soil drench inoculation allows more precise control of the number of Ph zoosporangia applied to a single sunower seedling. A detached leaf assay has been described, but it has been used mainly for Ph subcultivation and fungicide tests. For race determination, a differential set consisting of nine sunower genotypes has been used since 1988, coupled with a numerical triplet code for virulence phenotyping of Ph. The increasing variability in global Ph populations has demonstrated the inadequacy of the current set of differentials, and several researchers have proposed additional public lines as new differentials. Furthermore, bulk isolates may show different results in repeated tests, as Ph may contain genetically distinct zoospores within a single zoosporangium. For precise race determination, single zoosporangia or single zoospore isolates are advisable. However, due to low success of isolation, approximately 1 2%, this method cannot be applied in routine Ph race screening. Methods surveyed in this review have a broad spectrum of applications, including taxonomic studies.

Keywords: Helianthus annuus, host resistance, inoculation, pathogen race, sunower downy mildew, virulence formula

Introduction

The biotrophic oomycete *Plasmopara halstedii* (Ph) is a global threat to sunower (*Helianthus annuus*) as the causal agent of downy mildew (Gulya, 2007). Ph is native to North America (Delmotte *et al.*, 2008) where it was rst found by Halsted near Cambridge (MA, USA) on *Eupatorium purpureum* in 1876 and described later by Farlow (1883; for review see Virânyi & Spring, 2011). Downy mildew on cultivated sunowers was rst recorded in the USA in the 1920s (Young & Morris, 1927). Cultivation of open-pollinated sunower varieties that were highly susceptible to Ph, both in Europe and North America, helped the rapid spread of the disease (Virânyi, 2002; Sedlâřovâ *et al.*, 2013, 2016; Bân *et al.*, 2014; Anonymous, 2015; Virânyi *et al.*, 2015) and caused several severe outbreaks (Zimmer, 1971; Gore, 2009). Therefore sunower downy mildew has been declared a quarantine pathogen in the European Union since 1992 (Delmotte *et al.*, 2008).

Since the rst appearance of Ph races in the 1970s (Zimmer, 1974), breeders have developed sunower germplasm using single dominant resistance genes, coded as *Pl* genes (Vear *et al.*, 2008a). To date, almost 20 *Pl* genes have been discovered and subsequently incorporated into the crop (Hahn & Wieckhorst, 2010; Qi *et al.*, 2014, 2016; Virânyi *et al.*, 2015), thus providing broad variation of race-specic resistance to downy mildew (Tourvieille de Labrouhe *et al.*, 2008). Similar to other pathosystems, growing of new sunower hybrids inuences the pathogenic variability of Ph. Already more than 44 races of Ph have been recorded worldwide (Sedlâřovâ *et al.*, 2016). Monitoring of Ph populations, in turn, provides necessary data for the crop breeding programmes.

This goal cannot be achieved without accurate, reliable and repeatable screening methods, which are surveyed and described in this paper. Many methods of isolation, multiplication, maintenance and storage of Ph have been developed. In addition, there have been many methods proposed for Ph virulence determination and sunower

*E-mail: ales.lebeda@upol.cz

Published online 12 September 2016

resistance. This article reviews these methods, consolidates them and aims to propose the optimal methodology for both handling this pathogen and examining its interactions with host plants.

Characteristics of the Pathosystem *H. annuus* *P. halstedii*

Species concept and host range of*P. halstedii*

The species concept of Ph has evolved greatly since its initial description (Farlow, 1883) and there is still no perfectly clear and broadly accepted opinion (for review see Virányi & Spring, 2011). For the purposes of this review, a simplied Ph *sensu lato* and *sensu stricto* concept is introduced, as explained in the following text. This concept is based on the wide and narrow species concept described by Voglmayr (2008).

Ph*sensu lato*(Choi*et al.*, 2009a) represents a morphologically well-characterized organism capable of infecting more than 80 species of the Asteraceae family (Gulya *et al.*, 1997). Infections of Ph were described for hosts other than *H. annuus*, such as invasive *Ambrosia artemisiifolia*(Walcz*et al.*, 2000; Vajna, 2002),*Iva xanthifolia*(Gulya, 2002a),*Ageratum houstonianum*(Mattos *et al.*, 2006), cultivated *Rudbeckia fulgida* Goldsturm (Dankers *et al.*, 2004; Hong, 2006; Rivera *et al.*, 2014) and *R. fulgida* var. *speciosa* (Rivera *et al.*, 2015), cultivated*Coreopsis lanceolata*(Choi*et al.*, 2009b) or perennial sunower *Helianthus*×*laetiorus* (Spring *et al.*, 2003). Because the pathogen was determined according to its morphology in most cases, not always complemented by Kochs postulates, it is not denite that these infections were caused by Ph. For example, *Xanthium strumarium* was rst also considered a potential host of Ph (Novotelnova, 1962) but the pathogen was later described as*Plasmopara angustiterminalis*(Novotelnova, 1962; Komjâti *et al.*, 2007). A similar situation arose with*Arctotis*×*hybrida*and*Arctotheca calendula*, which were proven to be hosts of a new species, *Plasmopara majewskii*(Constantinescu & Thines, 2010) and not Ph.

The Ph *sensu stricto* concept is based on molecular studies which revealed that *Plasmopara* isolates from *Ambrosia artemisiifolia* are clearly distinguishable from other*Plasmopara*isolates originating from*H. annuus*or *X. strumarium*(Choi*et al.*, 2009a).

It is also important to emphasize that isolates originating from only a few wild species (*A. artemisiifolia*(Walcz*et al.*, 2000), *I. xanthifolia* (Gulya, 2002a), *H.*×*laetiorus* (Spring *et al.*, 2003) and *X. strumarium* (Komjâti *et al.*, 2007)) were capable of infecting differential lines of *H. annuus* (Virányi & Spring, 2011). Recently, Rivera *et al.* (2016) also showed that Ph isolates originating from*R. fulgida*are able to cross-infect sunower.

It is evident that the host range of Ph can be understood in different ways according to the Ph *s. s.* or *s. l.* species concept and the situation is signicantly complicated by the occurrence of cross-infections, host jumps and possible cryptic species hybridization, such as in the case of *Pseudoperonospora cubensis*/*Pseudoperonospora humuli* species (Runge *et al.*, 2011). Recently, a high quality draft genome of Ph was analysed and reported (Sharma *et al.*, 2015) that enables much better understanding of pathogen variation and host pathogen interactions.

Sunower biology and genetics of resistance to *P. halstedii*

Cultivated sunower, *H. annuus*, is an annual diploid plant,n=17 (Heiser*et al.*, 1969). The genus*Helianthus* comprises 49 species from which 12 species are diploid annuals (n=17) and 37 species are diploid, tetraploid or hexaploid perennials (Seiler, 1992; Schilling, 2006; Kantar *et al.*, 2015). Ph can infect all *Helianthus* species (Virányi & Spring, 2011), although resistance is far more prevalent among the perennial species. The rst resistance to downy mildew (Pl_1) was identied in sunower hybrid Advent at ICCPT, Romania, almost 50 years ago (Vranceanu & Stoenescu, 1970). Resistance is much more easily transferred from the diploid annual species to cultivated sunower, but breeders have succeeded in transferring *Pl* genes from polyploid perennial *Helianthus* species as well (Vear, 2016). Documented sources of Ph resistance from wild *Helianthus* include some populations of annuals *H. annus*, *H. argophyllus* and *H. praecox* subsp. *hirtus* and perennial species *H. grosseserratus*, *H. maximiliani*, *H. nuttallii*, *H. pauciorus* and *H. tuberosus* (Seiler, 1992). One of the rst hybrids resistant to Ph was produced by hybridization of cultivated sunower with *H. tuberosus*, which resulted in the discovery of the Pl_2 gene (for review see Sackston, 1992). Later, other *Pl* genes and quantitative trait loci (QTLs) have been detected and exploited for the production of resistant hybrids. Up to now, resistance genes have been obtained from wild *H. annuus*, *H. praecox*, *H. tuberosus* and *H. argophyllus* (Mulpuri *et al.*, 2009; Qi*et al.*, 2016).

Major gene resistance and quantitative resistance are the main mechanisms recognized in sunower. Most of the resistance mechanisms are described as a gene-for-gene interaction based on *Pl* dominant resistance genes in sunowers and corresponding *Avr*genes in the pathogen (Keen, 1990). Many *Pl* genes have been discovered (for review see Vear*et al.*, 2008a; Mulpuri*et al.*, 2009), mapped (for review see Hahn & Wieckhorst, 2010; Qi *et al.*, 2016) and used in resistance breeding (Liu *et al.*, 2012). These genes, although from different origins, are not usually inherited individually but seem to form at least ve independent clusters (Table 1) (Vear, 2010). Individual *Pl* genes provide resistance against individual, several or many Ph races (Hahn & Wieckhorst, 2010). For example, the Pl_1 gene confers resistance against race 100, the Pl_2 gene provides resistance against races with triplet codes beginning with 100 and 3xx, and Pl_8 and Pl_{Arg} genes provide resistance against all known races (Vear, 2010; Qi*et al.*, 2016). However, selection pressure-driven evolution of new Ph races that

Table 1 *Pl* gene clusters from the publicly available *Helianthus annuus* map of simple sequence repeat (SSR) or microsatellite markers (according to Vincour *et al.* (2012); Vear *et al.* (2008a); Liu *et al.* (2012); Qi *et al.* (2014, 2016))

Linkage group	Resistance gene	Effective against races
LG8	Pl_1	100
	Pl_2	100, 3xx[a]
	Pl_6	1xx, 3xx, 7x1, 7x2, 7x3
	Pl_7[b]	1xx, 3xx, 7x1, 7x2, 7x3
	Pl_{15}	All currently known races in NA and EU[c]
LG13	Pl_5	1xx, 31x, 32x, 33x, 7xx
	Pl_8	All currently known races in Europe[d]
LG1	Pl_{Arg}	All currently known races
	Pl_{13}	100, 300, 310, 330, 700, 710, 730, 731, 770
	Pl_{14}	?
	Pl_{16}	?
LG2	Pl_{18}	All currently known races
LG4	Pl_{17}	All currently known races

NA, North America; EU, European Union.
[a]Except for race 304 and 314 with Pl_2 in sunflower line PSC8.
[b]Valid only for Pl_7 in sunflower line 83RM.
[c]Races virulent on Pl_{15} have been found in Argentina.
[d]Races virulent on Pl_8 have been found in the US in 2015/16.

overcome *Pl* genes can make this type of resistance rather short-lived (Ahmed *et al.*, 2012). Therefore quantitative (non-race specic) resistance, connected with QTLs, is the second main mechanism of resistance. This type of resistance has been studied during the past decade primarily by French INRA researchers (Bert *et al.*, 2004; Tourvieille de Labrouhe *et al.*, 2008; Vear *et al.*, 2008b). As yet, there have been no public releases of such germplasm. However, in the absence of effective *Pl* genes, some commercial hybrids suffer more virulent attacks by Ph than others, so eld resistance probably exists in marketed material.

Due to the intensive and long-lasting research of *Helianthus* germplasm, numerous well-maintained lines can be accessed from germplasm collections. The two largest sunower germplasm collections are located in the USDA-National Plant Germplasm System (NPGS) in Ames (IA, USA) and in the N. I. Vavilov Research Institute of Plant Industry (VIR) in St Petersburg, Russia (Gulya *et al.*, 2010). The USDAs sunower gene bank contains *c.* 2500 accessions of cultivated sunower, plus 1659 accessions of the annual *Helianthus* and 889 accessions of perennial species (Kane *et al.*, 2013; L. Marek, North Central Regional Plant Introduction Station, Iowa State University and USDA ARS, Ames, USA, personal communication). Seeds of both cultivated and wild *Helianthus* accessions are available to researchers worldwide, free of charge. Over 2300 accessions of both cultivated sunowers and wild *Helianthus* accessions from 48 countries can be found in VIR (Gulya *et al.*, 2010). Distribution of seeds to researchers outside Russia is somewhat limited. Similarly, the set of 500 accessions of wild *Helianthus* species and 420 cultivated accessions (including 331 old open-pollinated varieties and a core collection of 96 inbred lines) are available from INRAs seed centres in France (Kane *et al.*, 2013). There are other smaller sunower seed collections in Argentina (INTA), Brazil (Embrapa), Canada (Agriculture and Agri-Food Canada), China (CGRIS), India (NBPGR), Serbia (Institute of Field and Vegetable Crops, Novi Sad) and Spain (CSIC) (Gulya *et al.*, 2010).

Germplasm collections of the USDA and VIR are great sources of material for breeding and future research. Growing genomic resources, seed collections open to the public, interesting ecology of various sunower species and rapidly evolving molecular tools prove that sunower is a suitable taxon to study various ecological and evolutionary traits (Kane *et al.*, 2013).

Intraspecic variability of *P. halstedii*

According to the best of the authors knowledge 44 different races of Ph have been determined so far (Gulya, 2007; Liu *et al.*, 2012; Virányi *et al.*, 2015; Sedlářová *et al.*, 2016). Research on Ph virulence variation started in the 1970s with the rst appearance of virulent races. Since then, many isolates, both from cultivated sunowers and from other hosts, were collected and put in herbaria and other collections. All kinds of bulk single zoosporangia or single zoospore isolates can be found in collections of F. Vear and D. Tourvieille de Labrouhe (INRA, UMR 1095, Domaine de Crouelle, Ave du Brezet, Clermont-Ferrand, France), INRA (LIPM, Castanet Tolosan, France), T. J. Gulya (USDA-ARS Northern Crop Science Laboratory, Fargo, North Dakota, USA), O. Spring (Institute of Botany, University of Hohenheim, Stuttgart, Germany), F. Virányi (until 2011) and R. Bân (PlasmoProtect and Plant Protection Institute, Szent Istvân University, Godollo, Hungary), or A. Lebeda and M. Sedlářová (Department of Botany, Palacký University in Olomouc, Olomouc, Czech Republic).

Considering difculties of the species denition, Ph *s. s.* can be understood as a group of isolates attacking cultivated *H. annuus* and other *Helianthus* species. This group of isolates expresses high virulence diversity when grown on various sunower genotypes (Vir ányi & Spring, 2011). The pathogen virulence prole (i.e. race) is determined by reactions of differential sunower lines (Gulya *et al.*, 1998; Tourvieille de Labrouhe *et al.*, 2000). However, this method provides partly subjective results, leading to many researchers attempting to develop tests based on molecular biology methods (for review see Virányi & Spring, 2011).

Borovkov & McClean (1993) used a method of restriction fragment length polymorphisms (RFLP) and proved the existence of randomly repeated sequences that can distinguish between some races of Ph. Random amplied polymorphic DNA (RAPD) PCR markers studied by Borovkova *et al.* (1992) were too sensitive and revealed differences even between isolates from the same virulence phenotype. However, in a study by Roeckel-Drevet *et al.* (2003), RAPD-based analysis detected

differences between isolates from distant geographical regions and showed low genetic variability among isolates in the same region, caused probably by bottleneck and founder effects. Intelmann & Spring (2002) performed RFLP studies of mitochondrial DNA and used simple sequence repeats and microsatellites as primers for the PCR. Several primer combinations were successful in differentiating between various eld isolates but any correlation of a specic amplication pattern with specic pathotype was not observed. Target region amplication polymorphism (TRAP) and 86 TRAP markers were capable of discriminating every isolate tested by Chen *et al.* (2005), but none of the markers was tightly associated with pathogenicity (i.e. race) or with the geographic origins of the isolates. Variability of large repeated elements in ITS2 regions successfully discriminated isolates from other Ph hosts, but did not reveal variability among isolates of different races originating from *H. annuus* (Spring *et al.*, 2006a). Large repeated elements in ITS2 regions of other downy mildew pathogens and other large subunit (LSU)-based markers are more suitable for phylogeny studies (Voglmayr *et al.*, 2004; Thines, 2007a,b) or for Ph detection in seeds (Ioos *et al.*, 2007). Single nucleotide polymorphisms (SNPs) and size variation in expressed sequence tags (EST) were studied by Giresse *et al.* (2007) who revealed 12 EST-derived markers displaying SNPs and insertion-deletion variations. Those 12 EST-derived markers were used in Ph genetic, aggressiveness and morphology variability studies and revealed ve multilocus genotypes. However, no correlation among genetic proles, pathogenic races or morphological characteristics of the isolates was observed. SNPs detected by those 12 EST-derived markers revealed that Ph was introduced into France several times (Delmotte *et al.*, 2008). They also elucidated that the rapid occurrence of new Ph races in France during the last 20 years was probably caused by hybridization and clonal evolution of introduced races (Ahmed*et al.*, 2012).

Great efforts have been made to nd convenient DNA regions that might be used for molecular-based race determination, but no reliable molecular-based method for Ph race determination has been developed yet. Any determination of Ph races using molecular methods and DNA markers cannot be applied routinely (Virányi & Spring, 2011). Thus, researchers and plant breeders currently still need to rely on the phenotype of the reaction of sunower differential lines to Ph as the only practical bioassay for pathogen race identication and denomination.

Development of race determination and nomenclature

Since the rst description of Ph pathogenic races in the 1970s (Orellana, 1970; Zimmer, 1974), neither uniform race nomenclature nor a standardized set of differentials had been available until the 1990s (Gulya, 2007). Only two races of Ph existed (or were distinguishable) in the 1970s: race 1, also referred to as the European race, and race 2, referred to as Red River Valley race, which was present in north-central USA and Canada (Sackston *et al.*, 1990; Gulya, 2007). Race 1 attacked only sunowers without any resistance genes and was the only known race in Europe until the late 1980s, whereas race 2 was able to overcome thePl_1 resistance gene. The situation became more complicated in the US with occurrence of new races (Gulya *et al.*, 1991b), but neither a standardized differential set nor nomenclature was available at that time. Races were named with sequential numbers according to the order of race detection. In 1980, in North Dakota, a new race appeared, later denominated as race 3 (Carson, 1981), capable of overcoming Pl_1, Pl_2 and Pl_4 genes (Gulya *et al.*, 1982); in 1985, race 4, capable of overcomingPl_1,Pl_2,Pl_3 andPl_5 genes, appeared in Minnesota (Gulya & Urs, 1985) and in 1988, race 5, overcoming Pl_1, Pl_2, Pl_3, Pl_4 and Pl_5, appeared in the USA during greenhouse experiments (Ljubich *et al.*, 1988). Race 6 was then detected in a French isolate collected in 1988 and race 7 was rst described in an isolate originating from Argentina (Gulya *et al.*, 1991b). The potential for a sequential numbering system to cause confusion was becoming apparent because the same number could be used for different races discovered in different countries (Gulya, 2007). In France, a new system based on alphabet letters was introduced for a brief time (Tourvieille de Labrouhe *et al.*, 2000).

There was a practical need to apply a different approach to race nomenclature, so Sackston*et al.*(1990) and Gulya *et al.* (1991a) proposed a new testing and denomination system based on virulence formulas using *Pl* genes overcome by the respective isolates. The virulence formulas of the rst known Ph races are listed in Table 2. This system, the rst attempt at standardization of an alternative to sequential numbering, proved impractical and ambiguous and was not accepted by researchers. The rst ofcial differential set (Table 3) was proposed, which laid the foundation of the differential set used recently (Gulya*et al.*, 1991a), but sequential numbers continued to be used, with the latest known sequential race number being 11 (Gulya, 2007).

As soon as pathogen race information exceeds a certain level of complexity, mathematical codes are most advantageous, in particular coded triplets. Thus, a triplet coding system was proposed in 1995 to ease communication and comparisons of results, based on a new differential set of nine sunower inbred lines available from the USDA (Gulya, 1995). These lines, and a triplet coding nomenclature system, were adopted by a multinational committee of pathologists and have been widely accepted globally. Races characterized prior to 1995 could be assigned a triplet code (i.e. race 1 became race 100, race 2 became 300), but in reality, a correct race designation for an isolate collected prior to 1995 could only be made if it was subsequently tested on the current nine differentials (Tourvieille de Labrouhe*et al.*, 2000; Gulya, 2007). This system has been used up to now with minor changes (Limpert & Muller, 1994; Gulya *et al.*, 1998; Tourvieille de Labrouhe*et al.*, 2000).

Table 2 Coded virulence formulas (CVF) of the sunflower differential set and names of the first known *Plasmopara halstedii* races (adopted according to Gulya, 2007)

		P. halstedii race										
Sequential number		1	2	3	4	5	6	7	8	9	10	11
Triplet CVF		100	300	700	730	770	310	330	710	330	703	731
Differential set[c]		Reaction of differential lines										
D-1	HA-304	s	s	s	s	s	s	s	s	s	s	s
D-2	RHA-265	R	s	s	s	s	s	s	s	s	s	s
D-3	RHA-274	R	R	s	s	s	R	R	s	R	s	s
D-4	PMI-3 (DM-2)	R	R	R	s	s	s	s	s	s	R	s
D-5	PM-17	R	R	R	s	s	R	s	R	s	R	s
D-6	803-1	R	R	R	R	s	R	R	R	R	R	R
D-7	HAR-4	R	R	R	R	R	R	R	R	R	s	s
D-8	QHP1 (HAR-5)	R	R	R	R	R	R	R	R	R	s	R
D-9	HA-335	R	R	R	R	R	R	R	R	R	R	R
French name[a]			D	C					A		B	
Pl genes		0 [b]	1[b] 1,5[c]	1,2[b] 1,2,3,4,b[c]	1,2,5 (CL)[b] 1,2,3,4,5,a,b,e[c]	1,2,5[b] 1,2,3,4,5,a,b,c,d,e[c]	1,9[b] 1,2,5,b[c]	1,2,5,c[c]	1,2,3,4,a,b[c]			

D-1 to D-9, lines of differential set. R, resistant; s, susceptible.
[a]Tourvieille de Labrouhe *et al.* (2000).
[b]Sackston *et al.* (1990).
[c]Gulya *et al.* (1991a).

The currently used differential sunower set (Table 2) consists of nine differential lines (D-1 to D-9), divided into three groups of three (Tourvieille de Labrouhe *et al.*, 2000) and that carry specic combinations of resistance genes (Table 1). Individual races are determined on the basis of the phenotypic response of the differential lines (DLs) from each triplet (Gulya *et al.*, 1998) and each triplet reaction phenotype represents one number in the three-number code used for race denomination. Thus, each DL is evaluated as either susceptible (s) or resistant (R) to a pathogen isolate. If a DL is evaluated as resistant, a value of 0 is given. On the other hand, if the DL is susceptible, a value is given according to its position in the triplet: if the DL is rst in the triplet a value of 1 is given, if second, it generates a value of 2, and if third, a value of 4 is given. These values are then added to generate the code number. For example, the race 714 is coded as follows: in the rst triplet all DLs are susceptible (s+s+s=1+2+4=7), in the second triplet the rst DL is susceptible, while the second and third DLs are resistant (s+R+R=1+0+0=1), in the third triplet the rst and second DLs are resistant, and the third DL is susceptible (R+R+s=0+0+4=4) (for other examples, see Tourvieille de Labrouhe *et al.*, 2000). However, recently, an extension of the triplet code to a quintuplet code was proposed, with 15 differentials recommended to be used for Ph race evaluation (Tourvieille de Labrouhe *et al.*, 2012). In this system Ph races are named by a ve-digit code (Gascuel *et al.*, 2015; Sedlářová *et al.*, 2016). Despite a long lasting discussion in the sunower community, the six additional lines still await international endorsement.

The triplet code serves two purposes, as it stands for the race nomenclature and also represents the coded virulence formula (CVF), which gives information about the virulence pattern of the race. The CVF is exible and the code can be extended with additional differentials (in groups of three) without losing information of the original CVF. This system has also been used for other pathogens, not only oomycetes (e.g. *Bremia lactucae* (Lebeda & Petrźelovâ, 2010)), but also fungi (e.g. rusts of cereals (Bushnell & Roelfs, 1984)).

The differentials used for Ph race identication have gone through several permutations since their introduction. The rst two sets, proposed by Sackston *et al.* (1990) and Gulya *et al.* (1991a), were not used for triplet code nomenclature, but only served for *Pl* gene virulence formula determination. The rst differential set designed for the triplet coding system of race determination, similar to one that is presently used, was introduced in 1995 and consisted entirely of USDA released lines (Gulya, 1995). Slight modications of the original set appeared in 1998, with the suggested replacement of two USDA lines with INRA reselections (Gulya *et al.*, 1998; Tourvieille de Labrouhe *et al.*, 2000). In some instances, researchers used closely related lines containing the same *Pl* genes as those in the original differential set (Table 3). In an effort to achieve global consistency in race identication, most researchers have adopted the lines proposed.

With the evolution of new virulent races overcoming all nine original differential lines (Ahmed *et al.*, 2012), it is apparent that additional differentials are needed (Tourvieille de Labrouhe *et al.*, 2012; Gascuel *et al.*, 2015). Both the USDA and INRA have developed germplasm

Table 3 Comparison of differential sets of sunflower lines used for determination of virulence formulas for races of*Plasmopara halstedii*

	Sackston*et al.*(1990)	Gulya*et al.* (1991a)	Mouzeyar *et al.*(1994)	Virányi & Gulya (1995)	Gulya (1995)	Molinero-Ruiz *et al.*(1998)	Rozynek & Spring (2000)	Gulya *et al.*(1998); Tourvieille de Labrouhe*et al.* (2000)	Molinero-Ruiz *et al.*(2002)	Shindrova (2005)	Tourvieille de Labrouhe *et al.*(2012)
Differential sets used in notable works of various authors											
D-1	Peredovik; Krasnodarets; IS-003; HA-8; HA-300	HA-300	HA-89	GK-70 Iregi; IS-223	**HA-821**	HA-821	HA-821	**HA-304**	Peredovik; IS-003; HA-304	AD 66	**GB**
D-2	CM 5RR; CM 90 RR; HA-60; R 18; RHA-265; RHA-266	RHA-266	RHA-266	AD-66; RHA-265	**RHA-265**	RHA-265	RHA-266	**RHA-265**	RHA-265; RHA-266	RHA-265	**RHA-265**
D-3	RHA-274; IS-7000	RHA-274 HA-61	RHA-274	RHA-274	**RHA-274**	RHA-274	RHA-274	**RHA-274**	RHA-274; HA-61	RHA-274	**RHA-274**
D-4	DM-2; DM-3; IS-2000; IS-3003	DM-2	PMI3	DM-2	**DM-2**	DM-2	DM-2	**PMI3**	DM-2; PMI3	PMI3	**PMI3**
D-5					**PM-17**	PM-17	799-2	**PM-17**	799-1	PM-17	**PM-17**
D-6				803-1	**803-1**	803-1	803-1	**803-1**	803-1	803-1	**803-1**
D-7		HAR-4	HAR-4		**HAR-4**	HAR-4	HAR-4	**HAR-4**	HAR-4	HAR-4	**HAR-4**
D-8		HAR-5	HAR-5; QHP1		**HAR-5**	HAR-5	HAR-5	**QHP1**	HAR-5; QHP1	HAR-5	**QHP2**
D-9	HA-335; HA-336; HA-337; HA-338; HA-340	HA-335		HA-335	**HA-335**	HA-335	HA-335	**HA-335**		HA-335	**HA-335**
D-10											**Y7Q**
D-11											**PSC8**
D-12											**XA**
D-13											**PSS2RM**
D-14											**VAQ**
D-15											**RHA-419**
Additional[a]	HA-61; HIR-34	HIR-34; RHA-325; DM-6; HA-337; RHA-340	HIR-34 XP; DM-3; HA-338	HIR-34; HA-61; RHA-325; IS-2000		RHA-295; RHA-325; RHA-340			RHA-325; 799-1; RHA-340		

The officially proposed and standardized differential sets are presented in bold.

D-1 to D-15, lines of differential set.

[a]Lines that have not been used in official differential sets, but were used by respective authors for supplementary information.

with Ph resistance and in some cases have identied new *Pl* genes. To be most useful to the global research community, any additional lines should meet several criteria. They should be homozygous, xed lines with a single, new gene. Lines with new *Pl* genes that are already used by private breeders in commercial hybrids should be considered over lines deemed less desirable by industry breeders. From an agronomic point of view they should be easy to grow in all production areas, be self-compatible, and produce moderate to large quantities of seed per plant. Additionally, the proposed lines should be freely available to public and private researchers without any restrictions, so that they could be used both for race identication and also for breeding purposes. In this way, ultimately, the new genes could be incorporated into commercial hybrids to aid growers to control downy mildew. An ideal scenario would be the development of isogenic lines containing the same genes as the original differential set, as achieved by Flor for testing the ax rust pathogen (Flor, 1955). By backcrossing the current differential lines with an inbred line having good agronomic characters, one could ensure easy seed production. Also, one could select for lines that allow high, uniform sporulation by races of Ph to which they are susceptible, which would make interpretation of the downy mildew reaction phenotype easier to assess.

Sunower downy mildew control

The most effective disease management relies upon the cultivation of certied seed of resistant sunower (Vear *et al.*, 1997). However, single race-specic resistance has been overcome by new races with more complex virulence emerging due to signicant selection pressure (Ahmed *et al.*, 2012). Therefore, growers in most countries also rely upon chemical control by seed-applied fungicides. The rst chemical control, both in the EU and the USA, was provided by systemic phenylamide seed treatments (Albourie*et al.*, 1998) such as metalaxyl, used since 1980 (Kiss*et al.*, 1979; Iliescu, 1980; Melero-Vara *et al.*, 1982), or mefenoxam introduced a decade later (Shetty, 1998). Nevertheless, since 1995, metalaxyl tolerant isolates have been discovered, both in the EU and the USA (Oros & Virányi, 1984; Delen*et al.*, 1985; Gulya *et al.*, 1999; Molinero-Ruiz *et al.*, 2005; Spring *et al.*, 2006b; Garcâ-Ruiz *et al.*, 2012) as well as a report of mefenoxam-tolerant isolates (Molinero-Ruiz *et al.*, 2005). Subsequently, different chemical compounds were tested (Gulya, 2002b; Sudisha *et al.*, 2010) and the strobilurin compound azoxystrobin (FRAC 11) is nowadays used for sunower seed dressing to prevent underground infection of seedlings in some countries. A new compound, oxathiapiprolin, has recently been found to be effective against oomycetes (Pasteris *et al.*, 2016). Its biological activity was tested against *Phytophthora infestans* and *Plasmopara viticola* but it might also have potential in sunower protection.

Application of plant growth-promoting fungi (PGPF) (Nagaraju *et al.*, 2012) and plant growth-promoting rhizobacteria (PGPR) (Nandeesh Kumar *et al.*, 2008) represent ecofriendly and promising strategies of disease management yet to be tested and evaluated. However, growing of hybrids with new dominant *Pl* genes or QTLs combined with other approaches of integrated crop protection remains one of the approaches promising sustainable sunower downy mildew control (Vear, 2016).

Methodology of Tests

Pathogen collection and isolation

While many researchers collect the entire downy mildew infected plant, only one or two infected leaves bearing abundant sporulation need to be collected to provide sufcient zoosporangia for race identication. The plant material should be kept in labelled, moisture-tight containers (plastic boxes or bags) and kept cool (Gulya *et al.*, 1991a). Collected material should be transported to the laboratory as soon as possible. Dirty, non-sporulating samples can be rinsed in running tap water, placed in containers with moist blotter paper, and incubated overnight in the dark at temperatures of 15 18 °C to induce sporulation. Because sporangia collected from the eld are of unknown age and viability, it is advisable to rst inoculate them onto a universal suscept (bearing no known resistance genes) to produce fresh sporangia with high viability (Gulya*et al.*, 1991a).

Individual Ph isolates can be obtained either from single, infected leaves (bulk isolates) or from single zoosporangia/zoospores and each type of isolate is useful for different purposes. The advantage of bulk isolates is that preparing them is relatively easy, fast and requires no special equipment. Bulk isolates also preserve the possible variability of the original sample and that is why they represent close imitation of the situation in natural conditions (Molinero-Ruiz *et al.*, 1998, 2002). Thus, those isolates are suitable for testing resistance or susceptibility of sunower germplasm to Ph in traditional plant breeding. At the same time, bulk isolates are probably genetically heterogeneous, and thus are not entirely appropriate for taxonomical or molecular biology studies.

Many different techniques of single spore isolation have been used for various fungi (Hildebrand, 1938; Ho & Ko, 1997; Goh, 1999). However, in general they are not applicable to biotrophic organisms. A useful method for isolating single zoosporangia or zoospores of Ph was developed by Sackston & Vimard (1988) and Spring *et al.*(1998), using sunower leaf disc inoculation (LDI) (Spring *et al.*, 1997). The efciency of single-zoosporangia inoculations is low (*c.* 10 15%, T. J. Gulya, unpublished data). However, isolation of a single zoosporangium does not guarantee genetic homogeneity, because each zoosporangium contains multiple motile zoospores (Spring *et al.*, 1998). Thus, single zoospore isolates would be most genetically uniform, but, as yet, there are no published methods to efciently produce such isolates. In cooperation with Professor O. Spring

(Hohenheim University, Germany), the authors have optimized a capillary tube technique for single zoospore isolation of*P. halstedii*. Fresh zoosporangia are rst cultivated on 1% water agar in the dark at 19 °C for *c.* 1 h. Released zoospores are then isolated under an inverted microscope using a capillary tube connected to a micromanipulator and transferred onto cotyledon segments from a susceptible sunower line. The inoculated cotyledons are incubated at 19°C with a 12/12 h (light/dark) photoperiod, omitting the initial dark period of 12 16 h. Leaf discs are best cultivated in cultivation plates containing distilled water, which provides humidity for later sporulation. To the authors knowledge, the efciency of single-zoospore inoculations is even lower than for zoosporangia (1 2%; O. Spring, Hohenheim University, Germany, personal communication; Z. Trojanovâ & M. Sedlâřovâ, unpublished data). Thus, this method is very time-consuming, and would be impractical if dealing with large numbers of isolates.

Preparation of plant material for infection

Cultivars with a high level of susceptibility to all known Ph races, and without resistance genes, are required for sunower downy mildew growth and multiplication. There are many possible cultivars that are suitable for Ph cultivation such as: HA-89 (Sackston *et al.*, 1990), HA-288 (T. J. Gulya, unpublished data), HA-300 (Gulya *et al.*, 1991b), HA-302 (Gulya, 1996), HA-304 (Tourvieille de Labrouhe *et al.*, 2012), HA-821 (Spring *et al.*, 1997), HA-850 (Gulya, 1996), Giganteus (Spring *et al.*, 1997), Peredovik, Krasnodarets (Sackston *et al.*, 1990), INRA inbred lines FU, BT, GB (Tourvieille de Labrouhe *et al.*, 2012) and AD-66 (Shindrova, 2005) or line GK-70, which is no longer available (Virânyi & Gulya, 1995). Public inbred lines are best suited, because seed can be easily produced, and are generally available well in to the future. In contrast, open-pollinated cultivars (e.g. Krasnodarets) and commercial hybrids are less desirable because they are only available for a limited time period and generally not available globally. When considering inbred lines, confection lines, because of their larger cotyledons, generally enable the production of more spores than the smaller-seed oilseed lines. Ph can be maintained either on seedlings or on plant parts such as detached leaves or leaf discs. Each method has its advantages and is convenient for different purposes.

The simplest method of obtaining a culture of Ph is to plant susceptible sunower cultivars in soil already containing Ph oospores that can be obtained from elds with a previous history of downy mildew. It is also possible to use Ph-contaminated elds directly for cultivation of susceptible sunower cultivars. However, these elds may not be conveniently close, and infection rates are unpredictable because infection is highly dependent on the presence of waterlogged soil soon after planting. This method cannot be recommended, because Ph is a quarantine disease in all countries of the European Union (Anonymous, 1997).

Whole seedlings or plant parts are suitable for Ph cultivation under laboratory conditions. Sunower seeds to be inoculated with Ph should be clean and contamination-free, which diminishes the risk of damping off during growth. Simple surface sterilization of seeds can be accomplished by immersing in a dilute solution of household bleach (0.5 1% sodium hypochlorite) plus a small amount of surfactant or dish detergent to aid seed wetting (Gulya, 1996). Seeds should be soaked for 2 15 min, but longer immersions have no adverse effect on seed viability, and may hasten seed germination due to rehydration. Afterwards, the seeds should be thoroughly rinsed under running tap water.

Germination of sterilized seed can be achieved in many ways. Seed can be spread in one layer on a tray with a wet germination paper or seed germination blotters placed both underneath and on top of the seed, and kept in a germinator (Gulya, 1996). Alternatively, seed can be germinated in ragdolls (Gulya, 1996), in which seed is spread in between two moistened layers of brown germination paper that is rolled up and placed in a plastic bag (F. Virânyi, Szent Istvân University, Godollo, Hungary, personal communication). Germination can also be carried out on moistened lter paper in a Petri dish (Carson, 1981; O. Spring, Hohenheim University, Germany, personal communication). Incubation of seed is performed in the dark, with temperatures varying from laboratory to laboratory (room temperature, or incubators set at 20 24 °C). The time from seed germination to optimal radicle length for inoculation is usually 2 3 days, depending on cultivar and temperature. According to the authors experience, germination of seed between two layers of moistened lter paper in a plastic bag at 20 °C produces seedlings suitable for inoculation within 3 days. It should be emphasized that different lines require different times from germination to reaching the appropriate size for inoculation. To get all differential lines ready for simultaneous inoculation, germination of cultivars with slow growth rates should be initiated one or more days earlier than fast-growing cultivars. Alternatively, seedlings of the appropriate radicle size can be selected and stored at 4 °C until slower-germinating seedlings have reached the proper stage.

Only healthy seedlings without any damage or signs of microbial contamination, with 1 2 cm long roots, should be used for inoculation. Removal of seed hulls is not necessary for whole seedling inoculation, as this does not decrease the infection rate (Gulya, 1996). Moreover, careless dehulling may damage the seedlings and lead to growth disorders or infection by secondary pathogens.

Inoculated seedlings can be planted into soil, sand, perlite or in various mixtures of these substrates. The growth substrate should be free of pathogens, well-drained and low in nitrogen (Gulya, 1996). Growing inoculated sunowers in soil represents the most natural conditions but there is a great risk of occurrence of other soil pathogens. Therefore sterilization of the soil (both eld and commercially available mixtures) is strongly recommended. Some types of soil and substrates contain

high amounts of clay or organic matter that make the growth medium susceptible to waterlogging. This may lead to damping off of seedlings, premature sporulation of isolates (and contamination with other strains) or can act antagonistically to downy mildew development. Gulya (1996) recommends a substrate consisting of washed ll sand and coarse grade perlite mixed in a ratio of 3:2 (v/v). This kind of substrate is relatively sterile, pathogen-free, well-drained and inexpensive. However, neither sand nor perlite contains any nutrients and so it is recommended to use a water-soluble fertilizer application once a week (Gulya, 1996). In the authors experience, the best substrate for these tests is moistened perlite, which is cheap, pathogen-free, not subject to waterlogging, and has the additional benet of being much lighter than the sand/perlite mixture. Disinfection of growing containers and other tools by 1% KOH is highly recommended to avoid risk of contamination by Plasmopara halstedii virus, which causes hypovirulence of the downy mildew (Grasse*et al.*, 2013).

*Plasmopara halstedii*inoculum preparation and inoculation

Inoculum can be prepared from either fresh or frozen sporangia. If possible, it is recommended to use fresh sporangia from greenhouse-grown plants (Gulya *et al.*, 1991a; Gulya, 1996). An alternative to fresh sporangia is sporangia stored directly on sunower leaves at 4 °C in moist conditions, which can be used for inoculation up to 7 days after sporulation induction (T. J. Gulya, unpublished data). Zoosporangia can be preserved for a longer time at =20 °C, =80 °C or in liquid nitrogen. Zoosporangia stored directly on detached cotyledons at =20°C remain viable up to 3 months (Vir ânyi, 1985). If stored at =80 °C they can survive up to 1 or several years, although the survival percentage declines with time (M. Sedlâŕovâ*et al.*, unpublished data). This is consistent with ndings of Vir ânyi (1985), who proved that zoosporangia on detached cotyledons survived at=60°C up to 1 year. Several private breeders in the USA routinely store sporulated leaves at =20 °C and are able to revive viable zoosporangia 8 12 months later (T. J. Gulya, unpublished data). This kind of storage is suitable for working collections and short-term storage. On the other hand, air-dried (<10% moisture) sporangia vacuumed from infected leaves and immediately frozen and stored in cryotubes in liquid nitrogen can be viable for up to a decade (Gulya *et al.*, 1993). In general, lower water content in the stored zoosporangia prolongs the time of possible storage (Gulya *et al.*, 1993). Therefore, vacuumed zoosporangia, dried over a desiccant and stored at =80 °C, would probably survive for many years also. Storage of zoosporangia dried to<10% moisture, either in liquid nitrogen or=80°C, are suitable for reliable long-lasting storage. However, inoculum resulting from such conservation should not be used directly. First, the isolate must be regenerated on a susceptible sunower genotype.

For inoculation, zoosporangia are rinsed off sporulated leaves and suspended in distilled water or a non-chlorinated spring water. While distilled water has been used from the start, recent research has shown that calcium ions are necessary for signalling with host plant and development of pathogenic oomycetes (i.e. *Pythium* and *Phytophthora*) (Donaldson & Deacon, 1992; Bircher & Hohl, 1999). Gulya (1996) found that a minimal amount of calcium (10 mM $CaCl_2$) greatly increases infection efciency, especially with low inoculum concentrations.

Zoosporangia concentration is determined with the aid of a haemocytometer or a counting chamber (Burker, Fuch-Rosenthal, etc.). Concentration of inoculum used by various researchers varies from *c.* 10 000 to 50 000 zoosporangia per millilitre. These concentrations usually provide up to 100% infection of plants, while higher concentration may cause seedling damping off (Gulya, 1996).

Sunower seedlings of appropriate size are inoculated either by immersion in inoculum (whole seedling inoculation, WSI) or by addition of appropriate amount of inoculum (soil drench inoculation, SDI) and incubating for 2 5 h in the dark at temperature ranging between 16 and 19°C. For details see below.

Cultivation of inoculated plants, pathogen sporulation

Inoculated sunowers are grown in suitable substrate medium in conditions optimal for Ph development as referred previously. Temperature favourable for Ph development is relatively low (16 20 °C) (Gulya *et al.*, 1991a; Gulya, 1996) with optimum at 19°C. Generally, higher temperatures (20 24°C) are appropriate for plant growth while lower temperatures (16 20°C) are favourable for pathogen development and sporulation. At 19°C both plant growth and pathogen sporulation will be satisfactory. Leaf discs should be cultivated at lower temperatures to avoid rotting and decomposition of the tissue before pathogen sporulation. Photoperiod should range from 12 to 16 h with approximate light intensity from 150 to 220μmol $m^{=2}$ $s^{=1}$ (Gulya *et al.*, 1991a). Inoculated seedlings should not be allowed to dry but, on the other hand, they should not be waterlogged for the entire time of cultivation, as anoxia induces stress, and thus the roots would be curtailed and systemic infection would not develop well (Gulya, 1996). Moreover, increased humidity might cause premature pathogen sporulation that could contaminate other Ph isolates grown at the same place. Plants should be watered daily and fertilized weekly with commercial general purpose fertilizer (i.e. 10-10-10) if grown in perlite or perlite sand mixture, but some laboratories omit this fertilization step. In USDA greenhouse tests, a water soluble fertilizer is used at a rate that supplies 100 ppm of N. Water level or moisture level should be checked daily in cultivation plates or trays with leaf discs and adjusted if needed.

Inoculated plants should be grown under the conditions mentioned above for 11 14 days or until rst true

leaves reach a length of 1 cm. At this stage symptoms of systemic infection should be already obvious and plant tissue is sturdy enough to survive pathogen sporulation. While infected plants may sometimes produce sporulation under greenhouse conditions, they usually require 100% relative humidity for at least 8 hours. This can be achieved by covering the plants with plastic bags or moving the plants to a chamber where misting is applied to achieve 100% relative humidity. Growing seedlings longer than 14 days may result in plant death as the systemic infection overwhelms the plant, which complicates matters by minimizing the amount of zoosporangia produced, which are used as inoculum for subsequent experiments. Ph will sporulate spontaneously on leaf discs after 5 10 days. It is important to point out that only plants inoculated with the same isolate should be housed in the same humidity chamber to induce sporulation, and thus minimize cross-contamination.

Virulence phenotype determination

There are three main methods that can be used for determining virulence phenotype WSI, SDI or LDI.

Whole seedling inoculation (described by Cohen & Sackston (1973)) testing is accomplished by inoculating germinated seedlings of the differential set. Plants must full the conditions described for subcultivation of Ph isolates and they are treated in the same way. WSI may be done several ways but generally it should be performed according to Gulya*et al.*(1991a). The inoculum should be prepared from fresh zoosporangia produced overnight and the concentration should be between 20 000 and 50 000 zoosporangia $mL^{=1}$. The seedlings of each differential line are immersed in inoculum in separate vials or small Petri dishes in the same way as seedlings of susceptible *H. annuus* cultivars during multiplication of the isolate. After a 3 h inoculation in the dark at 16 20 °C, 10 20 seedlings per differential line are planted in rows in a tray lled with suitable substrate. The inoculated plants are then cultivated in conditions suitable for Ph for 14 days. Although this method of differential set inoculation is precise and provides nearly 100% seedling infection, it requires a great amount of inoculum.

For SDI inoculation (Goossen & Sackston, 1968), at least 10 seedlings of suitable age, size and condition per differential line are planted in rows into a tray containing suitable substrate. Seedlings are placed just below the substrate surface so they are visible for inoculation. Inoculum is prepared and its concentration is determined using a counting chamber or haemocytometer. The volume containing approximately 10 000 zoosporangia is calculated and is deposited on each seedling using an automatic pipette. Trays with the inoculated differential set are then incubated for 12 h in the dark (covered with foil) and then maintained at normal greenhouse conditions. The phenotypes are assessed after 14 days. The advantage of this method is the very low amount of inoculum used and the reliability of the results compared to the previous method.

In the last method of virulence testing, LDI (Sackston & Vimard, 1988), leaf discs of approximately 12 mm in diameter are cut from cotyledons or true leaves of 14-day-old sunowers. These discs are immediately immersed into freshly prepared inoculum of concentration ranging between 20 000 and 50 000 zoosporangia $mL^{=1}$, and inoculated in the dark at 16 20 °C for 3 h. Inoculated leaf discs can be cultivated by oating in distilled water in appropriate closed sterile cultivation plates or placed on moistened cellulose cotton wool in a tray covered with glass. At least 15 leaf discs from each differential line are gently placed into rows, abaxial side up, in a tray containing moistened cotton wool covered by lter paper. Trays are then covered with glass to maintain high humidity and cultivated in conditions suitable for Ph. Leaf discs are evaluated at 5, 9, 12 and 15 dpi. LDI is not recommended for virulence phenotype testing as it produces false positive results (Spring *et al.*, 1997). The cut edge of the leaf disc might facilitate ingress into the tissue of a pathogen that would be stopped by plant barriers (cuticle, epidermis, etc.). Resistance mechanisms in sunower are thought to take place mostly in the root or hypocotyls, and thus the ability of a Ph isolate to infect leaf discs may not be a true reection of resistance/susceptibility. However, LDI is ideal for fungicide tests or clonal techniques (single zoosporangium/zoospore isolation).

Drawing from experience with the lettuce *Bremia lactucae*pathosystem, there are two basic methods (qualitative and quantitative) that can be used for assessing resistance/susceptibility of sunower to Ph (Lebeda & Petrźelovâ, 2010). Qualitative assessment is suitable for resistance screening of breeding material, whereas the quantitative method is more applicable to research purposes. The qualitative assessment scale in Table 4 is based on methods used for *B. lactucae* (Lebeda & Petrźelovâ, 2010) and can be used for both LDI and WSI.

For both methods of inoculation, the total degree of infection, which is expressed as a percentage of the maximum scores (Townsend & Heuberger, 1943), is determined for every tested line according to the equation:

Table 4 Scale for qualitative assessment of*Plasmopara halstedii* sporulation

Sign	Type of response	Characteristics
+	Susceptible	Macroscopically visible sporulation on 80 100% of seedlings
=	Resistant	No visible sporulation on seedlings
(=)	Incompletely resistant	Limited sporulation often followed by macroscopically visible chlorosis
(+)	Heterogeneous	Mixture of completely susceptible and some resistant plants in tested sample

$$P \quad \frac{\sum n \times v}{x \times N} \times 100$$

where P=the total degree of infection P, n=the number of leaf discs or seedlings in each assessed category (degree of infection), v=the degree of infection (0 3 for seedlings or 0 4 for leaf discs), x=scale range (3 for whole plants or 4 for leaf discs), N=total number of assessed plants or leaf discs (i.e. usually 15 leaf discs or 10 seedlings).

Interpretation of pathogenicity test results and evaluation of the intensity of pathogen sporulation is still very subjective, although there are semiquantitative evaluation systems that can help make evaluations more precise. Gulya *et al.* (1991a) did not fully describe resistant and susceptible reactions of inoculated plants, but only stated that profuse sporulation and stunting of plants are the main criteria for susceptibility. If a line is not 100% infected (in routine resistance tests, 90% is also acceptable in some laboratories), the inoculum is considered as a mixture of different races. In such cases, the pathogen should be subcultured from the differential lines giving anomalous results and supplemental tests with cultivated inoculum should be performed. If all lines exhibit <100% (or 90% in some laboratories) infection, then the test needs to be repeated in its entirety. In contrast, Tourvieille de Labrouhe *et al.* (2000) used a four-step scale. In this method, there are two resistant categories, RI=no sporulation and RII=weak sporulation on cotyledons; there are also two susceptible categories, SI=sporulation on leaves and SII=intense sporulation on cotyledons only. However, it is not clearly stated how many plants must be in categories SI and SII to interpret the line as susceptible. According to the present authors experience, intensity of pathogen sporulation on every leaf disc can be classed according to the semiquantitative 0 3 scale for whole plants (Dickinson & Crute, 1974) or 0 4 scale for leaf discs (Lebeda, 1983, 1986) (Table 5; Fig. 1).

At least 15 leaf discs or 10 seedlings in two replicates per isolate should be tested. The reaction of a particular differential line is then determined as either resistant or susceptible. If the total degree of infection P is lower than 30% and no leaf disc is classed in the 2, 3 or 4

Table 5 Semiquantitative scale (modified according to Lebeda, 1986) used for evaluation of *Plasmopara halstedii* sporulation intensity on sunflower differential lines

Category of sporulation intensity	Description of sporulation on specific plant material	
	Whole seedling	Leaf disc
0	No visible sporangiophores on leaves	No visible sporangiophores
1	Limited sporulation, sporadic sporangiophores present	≤25% of leaf disc surface is covered with sporangiophores
2	≤50% of cotyledon area covered with sporangiophores	25 50% of leaf disc surface is covered with sporangiophores
3	>50% of cotyledon area covered with sporangiophores	50 75% of leaf disc surface is covered with sporangiophores
4		>75% of leaf disc surface is covered with sporangiophores

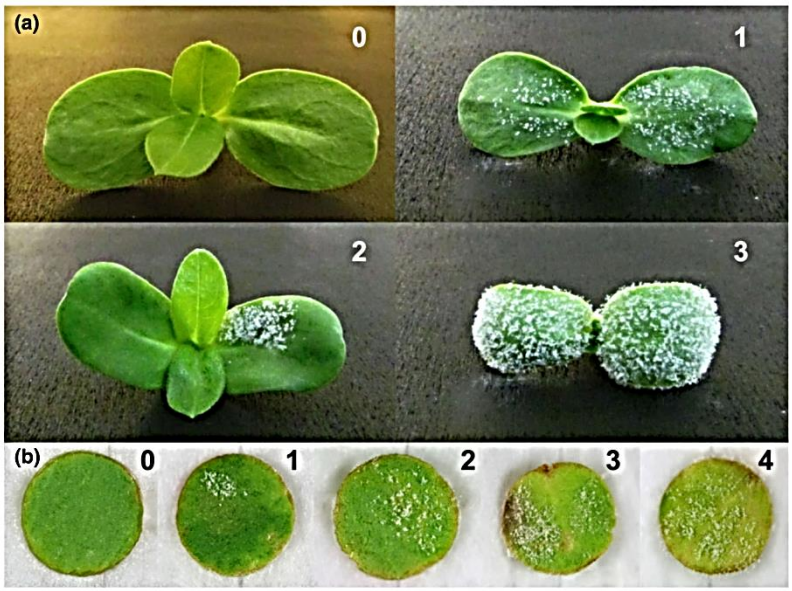


Figure 1 Semiquantitative scale for evaluation of the intensity of *Plasmopara halstedii* sporulation on sunflower differential lines during pathogenicity tests: (a) scale for whole seedling inoculation (0 3), (b) scale for leaf disc inoculation (0 4).

category of sporulation intensity, the differential line is considered to be resistant to the downy mildew; in the opposite case, the differential line is determined as susceptible. Even when applying these suggestions, some isolates are difcult to categorize and the result is somewhere between resistant and susceptible. In this case, it is recommended to repeat the whole procedure, but rst examine the purity of the seeds as well as the inoculum. The most precise method for virulence phenotyping would, ideally, be the use of monozoosporic isolates.

Conclusions

Methods for virulence phenotype (race) determination and denomination in Ph discussed here have a broad spectrum of applications, e.g. in searching for new sources of resistance in sunower germplasm, in resistance screening of new breeding material, and for detailed research into plant resistance mechanisms, virulence variation among Ph isolates and populations, and infection by Plasmopara halstedii virus. Although virulence phenotype evaluation still remains partly subjective, the previously described instructions can lead to reliable results. The most important points of methodology are: the use of the standardized set of differential lines, healthy sunower seedlings, growing in disinfected containers (to avoid contamination by Plasmopara halstedii virus), use of fresh Ph inoculum, and screening of disease symptoms on cotyledons as well as on the rst true leaves. These techniques are also the basic prerequisite for detailed genetic research, using either traditional methods or future molecular approaches. Half a century after the rst discovery of downy mildew resistance in sunower, research into Ph continues to be important and a topic for discussion between many laboratories around the world. The main goal of this review was to contribute to international harmonization of methodology used in Ph research and sunower breeding for downy mildew resistance.

Acknowledgements

The authors express their thanks to Professor F. Virányi and Dr R. Bân (Szent Istvân University, Godolo, Hungary), Professor O. Spring (University of Hohenheim, Germany) and to Dr Lili Qi (USDA-ARS, Fargo, ND, USDA) for cooperation with their laboratories. Comments of the reviewers were highly appreciated and improved the manuscript. Seeds of several sunower differential lines were kindly provided by Professor K. Veverka (Crop Research Institute in Prague, Czech Republic), and R. Zipper (University of Hohenheim, Germany). Excellent technical support for plant growth and propagation was provided by the authors colleagues D. Vondrâkovâ and J. Hanke, and students R. Pospĉhalov â and T. Bartŭšek. During the last decade, the work of Czech authors has been supported by the Ministry of Agriculture of the Czech Republic (project no. QH71254), Ministry of Education, Youth and Sports of the Czech Republic (project nos. MSM 6198959215, CZ.1.07/2.4.00/17.0007), and Palackŷ University in Olomouc (IGA_UP PrF-2016-001).

References

Ahmed S, Tourvieille de Labrouhe D, Delmotte D, 2012. Emerging virulence arising from hybridisation facilitated by multiple introductions of the sunower downy mildew pathogen *Plasmopara halstedii*.*Fungal Genetics and Biology***49**, 847 55.

Albourie JM, Tourvieille J, Tourvieille de Labrouhe D, 1998. Resistance to metalaxyl in isolates of the sunower pathogen *Plasmopara halstedii*.*European Journal of Plant Pathology***104**, 235 42.

Anonymous, 1997. Data sheets on quarantine pests*Plasmopara halstedii*. Prepared by CABI and EPPO for the EU under Contract 90/399003. [https://www.eppo.int/QUARANTINE/data_sheets/fungi/PLASHA_ds.pdf]. Accessed 29 February 2016.

Anonymous, 2015. Data sheet of*Plasmopara halstedii*(downy mildew of sunower). CABI Invasive Species Compendium. [http://www.cabi.org/isc/datasheet/41911]. Accessed 29 February 2016.

Bân R, Kovâcs A, Korosi K, Perczel M, Turôczi G, 2014. First report on the occurrence of a new pathotype, 714, of*Plasmopara halstedii* (sunower downy mildew) in Hungary. *Plant Disease***98**, 1580.

Bert P-F, Decham-Guillaume G, Serre F*et al.*, 2004. Comparative genetic analysis of quantitative traits in sunower (*Helianthus annuus* L.).*Theoretical and Applied Genetics***109**, 865 74.

Bircher U, Hohl HR, 1999. A role for calcium in appressorium induction in*Phytophthora palmivora*.*Botanica Helvetica***109** , 55 65.

Borovkov AY, McClean PE, 1993. A tandemly repeated sequence from the*Plasmopara halstedii*genome.*Gene***124**, 127 30.

Borovkova IG, Borovkov AY, McClean PE, Gulya TJ, Vick BA, 1992. Restriction fragment length polymorphisms and RAPD markers in DNA of*Plasmopara halstedii*, the downy mildew fungus of sunower. In:*Proceedings of 13th Sunower International Conference* . Pisa, Italy: Grache della Pacini, 1420 5.

Bushnell WR, Roelfs AP, 1984.*The Cereal Rusts: Origins, Specicity, Structure, and Physiology*. Orlando, USA: Academic Press.

Carson ML, 1981. New race of*Plasmopara halstedii*virulent on resistant sunowers in South Dakota. *Plant Disease***65** , 842 3.

Chen J, Gulya TJ, Bick BA, Hu J, 2005. TRAP analysis of molecular variability of a global collection of sunower downy mildew, *Plasmopara halstedii*. In: *Proceedings of Sunower Research Workshop*. Mandan, ND, USA: National Sunower Association, 12 3. [http://www.sunowernsa.com/uploads/research/210/Chen_TrapAnalysis_05.pdf]. Accessed 1 March 2016.

Choi YJ, Kiss L, Vajna L, Shin HD, 2009a. Characterization of *Plasmopara*species*Ambrosia artemisiifolia*, and notes on*Plasmopara halstedii*, based on morphology and multiple gene phylogenesis. *Mycological Research***113**, 1127 36.

Choi YJ, Park MJ, Shin HD, 2009b. First Korean report of downy mildew on*Coreopsis lanceolata*caused by*Plasmopara halstedii*.*Plant Pathology***58**, 1171.

Cohen Y, Sackston WE, 1973. Factors affecting infection of sunower by *Plasmopara halstedii*.*Canadian Journal of Botany***51** , 15 22.

Constantinescu O, Thines M, 2010. *Plasmopara halstedii* is absent from Australia and New Zealand. *Polish Botanical Journal* **55**, 293 8.

Dankers H, Kimbrough JW, Timur Momol M, 2004. First report of *Plasmopara halstedii*on perennial black-eyed susan in North Florida. *Plant Health Progress*. [https://www.plantmanagementnetwork.org/pub/php/brief/2004/susan/]. Accessed 1 March 2016.

Delen N, Onogur E, Yilz M, 1985. Sensitivity levels to metalaxyl in six *Plasmopara helianthi*Novot. isolates.*Journal of Turkish Phytopathology***14** , 31 6.

Delmotte F, Giresse X, Richard-Cervera S*et al.*, 2008. Single nucleotide polymorphisms reveal multiple introductions into France of

Plasmopara halstedii, the plant pathogen causing sunower downy mildew. *Infection, Genetics and Evolution* **8**, 534–40.

Dickinson CH, Crute IR, 1974. The inuence of seedling age and development on the infection of lettuce by *Bremia lactucae*. *Annals of Applied Biology* **76**, 49–61.

Donaldson SP, Deacon JW, 1992. Role of calcium in adhesion and germination of zoospore cysts of *Pythium*: a model to explain infection of host plants. *Microbiology* **138**, 2051–9.

Farlow WG, 1883. Note on some species in the third and eleventh centuries of Elliss North American Fungi. *Proceedings of the American Academy of Arts and Sciences* **18**, 71–3.

Flor HH, 1955. Host–parasite interaction in ax rust – its genetics and other implications. *Phytopathology* **45**, 680–5.

Garcâ-Ruiz R, L ôpez-Rivera S, Garcâ-Carneros AB, Molinero-Ruiz L, 2012. Field incidence of sunower downy mildew using seed dressing with metalaxyl-M. In: *Proceedings of the 18th International Sunower Conference*. Mar del Plata, Argentina. [http://www.asagir.org.ar/asagir2008/archivos_congreso/Field%20incidence%20of%20sunower%20downy%20mildew%20using%20seed%20dressing%20with%20metalaxyl-M.doc]. Accessed 2 August 2016.

Gascuel Q, Martinez I, Boniface M-C, Vear F, Pichon M, Godiard L, 2015. The sunower downy mildew pathogen *Plasmopara halstedii*. *Molecular Plant Pathology* **16**, 109–22.

Giresse X, Tourvieille de Labrouhe D, Richard-Cervera S, 2007. Twelve polymorphic expressed sequence tags-derived markers for *Plasmopara halstedii*, the causal agent of sunower downy mildew. *Molecular Ecology Notes* **7**, 1363–5.

Goh TK, 1999. Single spore isolation using a hand-made glass needle. *Fungal Diversity* **2**, 47–63.

Goossen PG, Sackston WE, 1968. Transmission and biology of sunower downy mildew. *Canadian Journal of Botany* **46**, 5–10.

Gore ME, 2009. Epidemic outbreaks of downy mildew caused by *Plasmopara halstedii* on sunower in Thrace, part of Marmara region of Turkey. *Plant Pathology* **58**, 396.

Grasse W, Zipper R, Totska M, Spring O, 2013. *Plasmopara halstedii virus* causes hypovirulence in *Plasmopara halstedii*, the downy pathogen of the sunower. *Fungal Genetics and Biology* **57**, 42–7.

Gulya TJ, 1995. Proposal of a revised system of classifying races of sunower downy mildew. In: *Proceedings of 17th Sunower Research Workshop*. Fargo, ND, USA: National Sunower Association, 76–8. [http://www.sunowernsa.com/uploads/research/686/1995_gulya_proposalrevisedsystem.pdf]. Accessed 29 February 2016.

Gulya TJ, 1996. Everything you should know about downy mildew but were afraid to ask. In: *Proceedings of 17th Sunower Research Workshop*. Fargo, ND, USA: National Sunower Association, 39–48. [http://www.sunowernsa.com/uploads/research/639/1996_gulya_everythingdowny.pdf]. Accessed 1 March 2016.

Gulya TJ, 2002a. First report of cross-infectivity of *Plasmopara halstedii* from Marshelder to Sunower. *Plant Disease* **86**, 919.

Gulya TJ, 2002b. Efcacy of single and two-way fungicide seed treatments for the control of metalaxyl-resistant strains of *Plasmopara halstedii* (sunower downy mildew). In: *Proceedings of the BCPC Conference Pests and Diseases*. Brighton, UK: BCPC, 575–80.

Gulya TJ, 2007. Distribution of *Plasmopara halstedii* races from sunower around the world. In: Lebeda A, Spencer-Phillips PTN, eds. *Advances in Downy Mildew Research, Vol 3. Proceedings of the 2nd International Downy Mildews Symposium at Palacký University in Olomouc and JOLA, Kostelec na Hané, Czech Republic*. Dordrecht, Netherlands: Springer, 121–34.

Gulya TJ, Urs RRNV, 1985. A new race of sunower downy mildew. *Phytopathology* **75**, 1339.

Gulya TJ, Carson ML, Urs RRNV, 1982. Race 3 sunower downy mildew: distribution and sources of resistance. *Phytopathology* **72**, 1136.

Gulya TJ, Miler JF, Virânyi F, Sackston WE, 1991a. Proposed internationally standardized method for race identication of *Plasmopara halstedii*. *Helia* **14**, 11–20.

Gulya TJ, Sackston WE, Virânyi F, Masirevic S, Rashid KY, 1991b. New races of the sunower downy mildew pathogen (*Plasmopara halstedii*) in Europe and North and South America. *Journal of Phytopathology* **132**, 303–11.

Gulya TJ, Masirevic S, Thomas CE, 1993. Preservation of air-dried downy mildew sporangia in liquid nitrogen without cryoprotectants or controlled freezing. *Mycological Research* **97**, 240–4.

Gulya TJ, Rashid KY, Masirevic SM, 1997. Sunower diseases. In: Schneiter AA, ed. *Sunower Technology and Production, No. 35. Agronomy*. Madison, WI, USA: American Society of Agronomy, 263–379.

Gulya TJ, Tourvieille de Labrouhe D, Masirevic S, Penaud A, Rashid K, Virânyi F, 1998. Proposal for standardized nomenclature and identication of races of *Plasmopara halstedii* (sunower downy mildew). In: Gulya TJ, Vear F, eds. *Proceedings of the International Sunower Association Symposium III, Sunower Downy Mildew*. Fargo, ND, USA: International Sunower Association, 130–6.

Gulya TJ, Draper M, Harbour J *et al.*, 1999. Metalaxyl resistance in sunower downy mildew in North America. In: *Proceedings of the 21st Sunower Research Workshop*. Bismarck, ND, USA: National Sunower Association, 118–23.

Gulya TJ, Marek LF, Gavrilova V, 2010. Disease resistance in cultivated sunower derived from public germplasm collections. In: *Breeding Sunower for Disease Resistance*. Krasnodar, Russia: International Sunower Association, [https://www.researchgate.net/publication/292148861_Disease_Resistance_in_Cultivated_Sunower_Derived_from_Public_Germplasm_Collections]. Accessed 4 July 2016.

Hahn V, Wieckhorst S, 2010. Mapping and tagging of simply inherited traits. In: Hu J, Seiler G, Kole C, eds. *Genetics, Genomics and Breeding of Sunower*. Eneld, NH, USA: Science Publishers, 111–34.

Heiser CB, Smith DM, Clevenger SB, Martin WM, 1969. The North American sunowers (*Helianthus*). *Memoirs of the Torrey Botanical Club* **22**, 1–218.

Hildebrand EM, 1938. Techniques for the isolation of single microorganisms. *The Botanical Review* **12**, 628–54.

Ho W-C, Ko W-H, 1997. A simple method for obtaining single-spore isolates of fungi. *Botanical Bulletin of Academia Sinica* **38**, 41–4.

Hong CX, 2006. Downy mildew of *Rudbeckia fulgida* cv. Goldsturm by *Plasmopara halstedii* in Virginia. *Plant Disease* **90**, 1461.

Iliescu H, 1980. La lutte contre le mildiou du tournesol par des tratements chimiques. In: *Proceedings IX Conferencia Internacional Del Girasol*. Torremolinos, Spain, 152–61. [http://isasunower.org/leadmin/documents/Proceedings/9thISC1980/1980Breeding/T1980BRE14_009.pdf]. Accessed 29 February 2016.

Intelmann F, Spring O, 2002. Analysis of total DNA by minisatellite and simple sequence repeat primers for the use of population studies in *Plasmopara halstedii*. *Canadian Journal of Microbiology* **48**, 555–9.

Ioos R, Laugustin L, Rose S, Tourvieille J, Tourvieille de Labrouhe D, 2007. Development of PCR test to detect the downy mildew causal agent *Plasmopara halstedii* in sunower seeds. *Plant Pathology* **56**, 209–18.

Kane NC, Burke JM, Marek L *et al.*, 2013. Sunower genetic, genomic and ecological resources. *Molecular Ecology Resources* **13**, 19–20.

Kantar MB, Sosa CC, Khoury CK *et al.*, 2015. Ecogeography and utility to plant breeding of the crop wild relatives of sunower (*Helianthus annuus* L.). *Frontiers in Plant Science* **6**. [http://journal.frontiersin.org/article/10.3389/fpls.2015.00841/full]. Accessed 29 February 2016.

Keen NT, 1990. Gene-for-gene complementarity in plant–pathogen interactions. *Annual Review of Genetics* **24**, 447–63.

Kiss G, Molnar J, Csete S, Galambos-Dienes J, 1979. A new possibility to control sunower downy mildew. *Növényvédelem* **15**, 27.

Komjâti H, Walcz I, Virânyi F, Zipper R, Thines M, Spring O, 2007. Characteristics of a *Plasmopara angustiterminalis* isolate from *Xanthium strumarium*. *European Journal of Plant Pathology* **119**, 421–8.

Lebeda A, 1983. Resistenzquellen gegenuber *Bremia lactucae* bei Wildarten von *Lactuca* spp. *Tagungsberichte – Deutsche Akademie der Landwirtschaftswissenschaften DDR. Berlin* **216**, 607–16.

Lebeda A, 1986. Specicity of interactions between wild *Lactuca*species and*Bremia lactucae*isolates from*Lactuca serriola*.*Journal of Phytopathology***117**, 54–64.

Lebeda A, Petrźelovâ I, 2010. Screening for resistance to lettuce downy mildew (*Bremia lactucae*). In: Spencer MM, Lebeda A, eds.*Mass Screening Techniques for Selecting Crops Resistant to Diseases*. Vienna, Austria: International Atomic Energy Agency, 245–56.

Limpert E, Muller K, 1994. Designation of pathotypes of plant pathogens.*Journal of Phytopathology***140**, 346–58.

Liu Z, Gulya TJ, Seiler GJ, Vick BA, Jan C-C, 2012. Molecular mapping of thePl_{16} downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in sunower. *Theoretical and Applied Genetics***125**, 121–31.

Ljubich A, Gulya TJ, Miller JF, 1988. A new race of sunower downy mildew in North America.*Phytopathology***78**, 1580.

Mattos JKA, Pio BLA, Gouveia J, Inacio C, Dianese JC, 2006. Primero relato de*Plasmopara halstedii*em*Ageratum houstonianum* (Asteraceae) no distrito federal, Brasil.*Fitopatologia Brasileira***31**, 413.

Melero-Vara JM, Garcia-Baudin C, Lopez-Herrera CJ, Jimenez-Diaz RM, 1982. Control of sunower downy mildew with metalaxyl. *Plant Disease***66**, 132–5.

Molinero-Ruiz L, Melero-Vara JM, Gulya TJ, 1998. Pathogenic characterization of*Plasmopara halstedii*isolates from Spain. In: Gulya TJ, Vear F, eds.*Proceedings of the International Sunower Association Symposium III, Sunower Downy Mildew*. Fargo, ND, USA: International Sunower Association, 26–9.

Molinero-Ruiz ML, Domĥguez J, Melero-Vara JM, 2002. Races of isolates of*Plasmopara halstedii*from Spain and studies on their virulence.*Plant Disease***86**, 736–40.

Molinero-Ruiz ML, Dominguez J, Gulya TJ, Melero-Vara JM, 2005. Reaction of eld populations of sunower downy mildew (*Plasmopara halstedii*) to metalaxyl and mefenoxam.*Helia***28**, 66–74.

Mouzeyar S, Delabrouhe DT, Vear F, 1994. Effect of host race combination on resistance of sunower, *Helianthus annuus*L., to downy mildew*Plasmopara halstedii*.*Journal of Phytopathology***141**, 249–58.

Mulpuri S, Liu Z, Feng J, Gulya TJ, Jan C-C, 2009. Inheritance and molecular mapping of a downy mildew resistance genePl_{13} in cultivated sunower (*Helianthus annuus*L.).*Theoretical and Applied Genetics***119**, 795–803.

Nagaraju A, Murali M, Sudisha J, Amruthesh KN, Mahadeva Murhty S, 2012. Benecal microbes promote plant growth and induce systemic resistance in sunower against downy mildew disease caused by *Plasmopara halstedii*.*Current Botany***3**, 12–8.

Nandeesh Kumar P, Ramachandra Kini K, Prakash HS, Niranjana SR, Shekar Shetty H, 2008. Induction of resistance against downy mildew on sunower by rhizobacteria. *Journal of Plant Interactions***3**, 255–62.

Novotelnova NS, 1962.*Plasmopara halstedii*as a composite species (the basis for the taxonomic division of the genus*Plasmopara*on Compositae).*Botanical Zhurnal (Moscow & Leningrad)***47**, 970–81.

Orellana RG, 1970. Resistance and susceptibility to downy mildew and variability in*Plasmopara halstedii*.*Bulletin of the Torrey Botanical Club***97**, 91–7.

Oros G, Virânyi F, 1984. Resistance of*Plasmopara halstedii*to metalaxyl in the greenhouse.*Temperate Downy Mildews Newsletter* **3**, 22–3.

Pasteris RJ, Hanagan MA, Bisaha JJ*et al*., 2016. Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein.*Bioorganic & Medicinal Chemistry***24**, 354–61.

Qi LL, Long YM, Jan CC, Ma GJ, Gulya TJ, 2014.Pl_{17} is a novel gene independent of known downy mildew resistance genes in the cultivated sunower (*Helianthus annuus*L.).*Theoretical and Applied Genetics* **128**, 757–67.

Qi LL, Foley ME, Cai XW, Gulya TJ, 2016. Genetics and mapping of a novel downy mildew resistance gene,Pl_{18}, introduced from wild *Helianthus argophyllus*into cultivated sunower (*Helianthus annuus* L.).*Theoretical and Applied Genetics***129**, 741–52.

Rivera Y, Rane K, Crouch JA, 2014. First report of downy mildew caused by*Plasmopara halstedii*on Black-eyed Susan (*Rudbeckia fulgida*cv. Goldsturm) in Maryland. *Plant Disease***98**, 1005.

Rivera Y, Creswell TC, Ruhl G, Crouch JA, 2015. First report of downy mildew caused by*Plasmopara halstedii*on native*Rudbeckia fulgida* Aiton var.*speciosa*(Wender.) Perdue in Indiana.*Plant Disease***99**, 1278.

Rivera Y, Salgado-Salazar C, Gulya TJ, Crouch JA, 2016. Newly emerged populations of*Plasmopara halstedii*infecting rudbeckia exhibit unique genotypic proles and are distinct from sunower-infecting strains.*Phytopathology***106**, 752–61.

Roeckel-Drevet P, Tourvieille J, Gulya TJ, Charmet G, Nicolas P, Tourvieille de Labrouhe D, 2003. Molecular variability of sunower downy mildew,*Plasmopara halstedii*, from different continents. *Canadian Journal of Microbiology***49**, 492–502.

Rozynek B, Spring O, 2000. Pathotypes of sunower downy mildew in southern parts of Germany.*Helia***23**, 27–34.

Runge F, Choi YJ, Thines M, 2011. Phylogenetic investigations in the genus*Pseudoperonospora*reveal overlooked species and cryptic diversity in the*P. cubensis*species cluster.*European Journal of Plant Pathology***129**, 135–46.

Sackston WE, 1992. On a treadmill: breeding sunowers for resistance to disease.*Annual Review of Phytopathology***30**, 529–51.

Sackston WE, Vimard B, 1988. Leaf disc immersion (LDI) inoculation of sunower with *Plasmopara halstedii*for*in vitro*determination of host pathogen relationships.*Plant Disease***72**, 227–9.

Sackston WE, Gulya TJ, Miller JF, 1990. A proposed international system of designating races of*Plasmopara halstedii*.*Plant Disease***74**, 721–3.

Schilling EE, 2006. 299.*Helianthus*. In: Flora of North America Editorial Committee, eds.*Flora of North America, Vol. 21. Magnoliophyta: Asteridae, Part 8: Asteraceae, Part 3*. New York, NY, USA: Oxford University Press, 141–69.

Sedlâřovâ M, Trojanovâ Z, Lebeda A, 2013. Distribution and harmfulness of*Plasmopara halstedii*on sunower in the Czech Republic.*Plant Protection Science***49**, 1–10.

Sedlâřovâ M, Pospĉhalov â R, Drâbkovâ Trojanovâ Z, Bartũšek T, Slobodianovâ L, Lebeda A, 2016. First report of*Plasmopara halstedii* new races 705 and 715 on sunower from Czech Republic. *Plant Protection Science***52**, 182–7.

Seiler GJ, 1992. Utilization of wild sunower species for the improvement of cultivated sunower. *Field Crops Research***30**, 195–230.

Sharma R, Xia X, Cano LM*et al*., 2015. Genome analyses of the sunower pathogen *Plasmopara halstedii*provide insights into effector evolution in downy mildews and*Phytophthora*.*BMC Genomics***16**, 741.

Shetty K, 1998. Managing metalaxyl-resistance in*Plasmopara halstedii* from a chemical company perspective. In: Gulya TJ, Vear F, eds. *Proceedings of the International Sunower Association Symposium III, Sunower Downy Mildew*. Fargo, ND, USA: International Sunower Association, 74–8.

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

Spring O, Rozynek B, Zipper R, 1997. Leaf disc inoculation–a useful tool for selecting infections of sunower downy mildew at low inoculum concentration, but inappropriate to pathotype characterization.*Journal of Phytopathology***145**, 189–91.

Spring O, Rozynek B, Zipper R, 1998. Single spore infections with sunower downy mildew. *Journal of Phytopathology***146**, 577–9.

Spring O, Voglmayr H, Riethmuller A, Oberwinkler F, 2003. Characterization of a*Plasmopara*isolate from*Helianthus×laetiorus* based on cross infection, morphological, fatty acids and molecular phylogenetic data.*Mycological Progress***2**, 163–70.

Spring O, Bachofer M, Thines M, Riethmuller A, Goker M, Oberwinkler F, 2006a. Intraspecic relationship of *Plasmopara halstedii*isolates differing in pathogenicity and geographic origin based on ITS sequence data.*European Journal of Plant Pathology***114**, 309–15.

Spring O, Zipper R, Heller-Dohmen M, 2006b. First report of metalaxyl resistant isolates of *Plasmopara halstedii* on cultivated sunower in Germany. *Journal of Plant Diseases and Protection* **113**, 224.

Sudisha J, Niranja SR, Sukanya SL, Girijamba R, Lakshmi Devi N, Shekar Shetty H, 2010. Relative efcacy of strobilurin formulations in the control of downy mildew of sunower. *Journal of Pest Science* **83**, 461 70.

Thines M, 2007a. Characterisation and phylogeny of repetitive elements giving rise to exceptional length of ITS2 in several downy mildew genera (Peronosporaceae). *Fungal Genetics and Biology* **44**, 199 207.

Thines M, 2007b. Repeats in the ITS2 of *Plasmopara* species and their relevance for phylogenetic studies. In: Lebeda A, Spencer-Phillips PTN, eds. *Advances in Downy Mildew Research, Vol 3. Proceedings of the 2nd International Downy Mildews Symposium*. Kostelec na Hanê, Czech Republic: Palackÿ University in Olomouc and JOLA; Springer Science and Business Media, 31 5.

Tourvieille de Labrouhe D, Gulya T, Maśirevic S, Penaud A, Rashid K, Virânyi F, 2000. New nomenclature of races of *Plasmopara halstedii* (sunower downy mildew). In: *Proceedings of the 15th International Sunower Conference* . Toulouse, France: International Sunower Association, I 61 6.

Tourvieille de Labrouhe D, Serre F, Walser P, Roche S, Vear F, 2008. Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunower (*Helianthus annuus*). *Euphytica* **164**, 433 44.

Tourvieille de Labrouhe D, Walser P, Jolivot D *et al.*, 2012. Proposal for improvement of sunower downy mildew race nomenclature. In: *Proceedings of the 18th International Sunower Conference* . Mar del Plata, Argentina. [https://www.researchgate.net/publication/283266439_Proposal_for_improvement_of_sunower_downy_mildew_race_nomenclature]. Accessed 1 March 2016.

Townsend GR, Heuberger W, 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter* **27**, 340 3.

Vajna L, 2002. Downy mildew epidemic on common ragweed in Hungary caused by *Plasmopara halstedii*. *Plant Pathology* **51**, 809.

Vear F, 2010. Classic genetics and breeding. In: Hu J, Seiler G, Kole C, eds. *Genetics, Genomics and Breeding of Sunower* . Eneld, NH, USA: Science Publishers, 51 78.

Vear F, 2016. Changes in sunower breeding over the last fty years. *Oilseeds and Fats Crops and Lipids* **23**, D202.

Vear F, Gentzbittel L, Philippon J *et al.*, 1997. The genetics of resistance to ve races of downy mildew (*Plasmopara halstedii*) in sunower (*Helianthus annuus* L.). *Theoretical and Applied Genetics* **95**, 584 9.

Vear F, Serieys H, Petit A *et al.*, 2008a. Origins of major genes for downy mildew resistance in sunower. In: *Proceedings of 17th International Sunower Conference* . Cordoba, Spain: Consejeria de Agricultura y Pesca, 125 30.

Vear F, Serre F, Jouan-Dufournel I *et al.*, 2008b. Inheritance of quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunower (*Helianthus annuus* L.). *Euphytica* **164**, 561 70.

Vincourt P, As-Sadi F, Bordat A, *et al.*, 2012. Consensus mapping of major resistance genes and independent QTL for quantitative resistance to sunower downy mildew. *Theoretical and Applied Genetics* **125**, 909 20.

Virânyi F, 1985. A simple technique for long-term storage of *Plasmopara halstedii* sporangia at low temperature. *Transactions of the British Mycological Society* **85**, 529 31.

Virânyi F, 2002. The sunower *Plasmopara halstedii* pathosystem: natural and articially induced coevolution. In: Spencer-Phillips PTN, Gisi U, Lebeda A, eds. *Advances in Downy Mildew Research*. Dordrecht, Netherlands: Springer, 167 72.

Virânyi F, Gulya TJ, 1995. Inter-isolate variation for virulence in *Plasmopara halstedii* (sunower downy mildew) from Hungary. *Plant Pathology* **44**, 619 24.

Virânyi F, Spring O, 2011. Advances in sunower downy mildew research. *European Journal of Plant Pathology* **129**, 207 20.

Virânyi F, Gulya TJ, Tourvieille de Labrouhe D, 2015. Recent changes in the pathogenic variability of *Plasmopara halstedii* (sunower downy mildew) populations from different continents. *Helia* **38**, 149 62.

Voglmayr H, 2008. Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology. *European Journal of Plant Pathology* **122**, 3 18.

Voglmayr H, Riethmuller A, Goker M, Weiss M, Oberwinkler F, 2004. Phylogenetic relationships of *Plasmopara*, *Bremia* and other genera of downy mildew pathogens with pyriform haustoria based on Bayesian analysis of partial LSU rDNA sequence data. *Mycological Research* **108**, 1011 24.

Vranceanu V, Stoenescu F, 1970. Immunity to sunower downy mildew due to a single dominant gene. *Probleme Agriculture* **22** , 34 40.

Walcz I, Bogâr K, Virânyi F, 2000. Study on *Ambrosia* isolate of *Plasmopara halstedii*. *Helia* **23** , 19 24.

Young PA, Morris HE, 1927. *Plasmopara* downy mildew of cultivated sunowers. *American Journal of Botany* **14**, 551 3.

Zimmer DE, 1971. A serious outbreak of downy mildew in principal sunower production in the United States. *Plant Disease Reporter* **55**, 11 2.

Zimmer DE, 1974. Physiological specialization between races of *Plasmopara halstedii* in America and Europe. *Phytopathology* **64**, 1465 7.

4.2. Geographical distribution of *Plasmopara halstedii*

4.2.1. Sedlářová, M., Trojanová, Z., Lebeda A., (2013): Distribution and harmfulness of *Plasmopara halstedii* on sunflower in the Czech Republic. *Plant Protection Science* 49(1), 1–10.

Distribution and Harmfulness of *Plasmopara halstedii* on Sunflower in the Czech Republic

Michaela SEDLÁŘOVÁ, Zuzana TROJANOVÁ and Aleš LEBEDA

Department of Botany, Faculty of Science, Palacký University in Olomouc, Olomouc, Czech Republic

Abstract

Sedlářová M., Trojanová Z., Lebeda A. (2013): **Distribution and harmfulness of *Plasmopara halstedii* on sunflower in the Czech Republic**. Plant Protect. Sci., **49**: 1–10.

In 2007–2012 the first detailed study of *P. halstedii* distribution and severity was performed in the Czech Republic by monitoring altogether 128 localities. Incidence of the pathogen was recorded at seven localities in south-eastern (Central and South Moravia) and central parts (East and Central Bohemia) of the country; at only four localities the occurrence of sunflower downy mildew symptoms and pathogen sporulation were recorded repeatedly. In all cases the primary infection prevailed, and the severity of infections was rather low. The majority of records were for sunflower experimental fields (e.g. fields of Central Institute for Supervising and Testing in Agriculture. Brno-Chrlice, Czech Republic), one record came from a commercial field and one record from a hobby field. Results of surveys indicate that reservoirs of primary inoculum likely exist in the soil contaminated by *P. halstedii* oospores in these habitats, but additional transfer by infected seed may be possible. Historical and geographical consequences of recorded infections are discussed, as well as virulence variation in the Czech pathogen populations which is currently in the process of investigation.

Keywords: sunflower downy mildew; *Helianthus annuus*; disease incidence; disease severity; pathogen population; quarantine disease

Sunflower downy mildew represents an economically important disease reducing the crop yield in all sunflower growing areas worldwide (Gulya 2007). The disease with typical symptoms, including damping off, dwarfing or retardation of flowering in systemically infected plants or local chlorotic spots on plants with secondary infection, is caused by the biotrophic oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni 1888 (originally described as *Peronospora halstedii* Farlow in 1882).

Sunflower is native to Central America and archaeobotanists showed that the crop was domesticated in the east-central United States (Heiser 2008). Although an additional domestication event in the north of Mexico was discussed, it has not been sufficiently proved by molecular and other data (Lentz *et al.* 2008 and responding articles in PNAS). According to previous knowledge the centre of *P. halstedii* origin was placed to North America (Sackston 1981). Probably the pathogen was later spread in a plant material to other continents, excluding Australia and Antarctica (Gulya 2007). *P. halstedii* as the causal agent of sunflower downy mildew was first described in the USA in 1888 (Šindelková *et al.* 2008). The first appearance of the disease in Europe was recorded in Russia in the 1960s (Delmotte *et al.* 2008). Later it expanded from Russia to the

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH71254, Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6198959215, and Palacký University in Olomouc, Project No. IGA UP PrF-2011-003.

Balkan Peninsula (Bulgaria, Greece, and Turkey) and consequently to many other European countries (e.g. Hungary, Italy, France, Spain, Germany, and the Czechoslovakia) (Virányi 2002). Intensive agriculture combined with suitable weather led to local epidemics of the disease throughout Europe in the second half of the 20th century. Since 1977 the spread of the disease has become serious enough to claim *P. halstedii* a dangerous harmful organism and threat to sunflower producers in Europe. Since 1992 *P. halstedii* has been a subject of quarantine regulation in the European Union (Delmotte *et al.* 2008). The paradigm that economically and epidemiologically relevant sunflower downy mildew incidences are derived from the only soil infections has been questioned (Spring 2009). Recently, a high potential for transition from local to systemic infection was found in field experiments with ornamental sunflower in Germany. These infections play a key role in the production of contaminated seeds carrying the pathogen into the next season (Spring 2009). While the local transfer of the disease is promoted by wind-borne sporangia or contaminated soil, its distinct transfer or introduction into new continents was probably enabled by seed containing the *P. halstedii* mycelium (Virányi & Spring 2011). The way of disease transfer by seed has been broadly discussed (Döken 1989).

The first occurrence of *P. halstedii* within the area of the former Czechoslovakia was reported in the 1950s by Bojňanský (1956, 1957) mainly from South Moravia and South Slovakia. Sunflower downy mildew was later found in experimental fields of Mendel University in Brno in 1999 (Veverka & Křížková-Kudlíková 2006). Since then only several observations of sunflower downy mildew were made and the distribution of the disease in the Czech Republic is considered to be rare (Kokeš & Müller 2004). This is also supported by the monitoring of harmful organisms done by State Phytosanitary Administration which proved the incidence of *P. halstedii* in the Czech Republic yearly since 2000, except for 2004 (State Phytosanitary Administration 2012). During the above-mentioned period *P. halstedii* was detected on eight localities in South Moravia (Tesařová *et al.* 2010a,b; State Phytosanitary Administration 2012), two localities in East Bohemia (State Phytosanitary Administration 2012) and one locality in West Moravia in 2011 (Fialová & Čech 2011).

Sunflower downy mildew has not been implicated in the main economic losses of sunflower yield in the Czech Republic while the most serious diseases include white mould caused by *Sclerotinia sclerotiorum* (Lib.) de Bary and grey mould caused by *Botrytis cinerea* Pers. that deteriorate the quality of the crop (Kudlíková & Veverka 1999). Since 2002 the sunflower downy mildew has been registered as quarantine disease in the Czech Republic and its occurrence has been monitored by State Phytosanitary Administration. Only resistance-certified and pathogen-free seed is allowed to be grown in the Czech Republic, and adherence to phytosanitary as well as agronomical practice is required (Šindelková *et al.* 2008).

Distribution of sunflower downy mildew and its race spectrum in the local pathogen populations have been intensively studied in several countries in Europe, such as Bulgaria (Shindrova 2010), Spain (Molinero-Ruiz *et al.* 2002), France (Sakr 2009), Germany (Rozynek & Spring 2000), Hungary (Virányi & Gulya 1995), and Italy (Tosi & Beccari 2007). However, no detailed information has been available for the Czech Republic. Although sunflower is not cultivated in the CR as extensively as in South-European countries and downy mildew is not the main threat to the crop, the position of this country in the Central European region creates conditions for the disease spread. Thus detailed and reliable information about the pathogen distribution in the Czech Republic was required.

The major objective of this paper is to present data from six-year systematic observations of the distribution and disease severity of *P. halstedii* on sunflowers grown in various areas and habitats within the Czech Republic.

MATERIAL AND METHODS

The occurrence of *Plasmopara halstedii* (Farl.) Berl. et de Toni 1888 was surveyed in the period of May–October in 2007–2011 and May–July in 2012 all over the Czech Republic with the main focus on the region of Central and South Moravia, the south-eastern part of the country, where sunflower (*Helianthus annuus* L.) is grown the most frequently, and on the region of East and Central Bohemia (Figure 1). At some localities the monitoring was repeated annually in order to determine changes in the incidence of the infection. Several characteristics were recorded at each

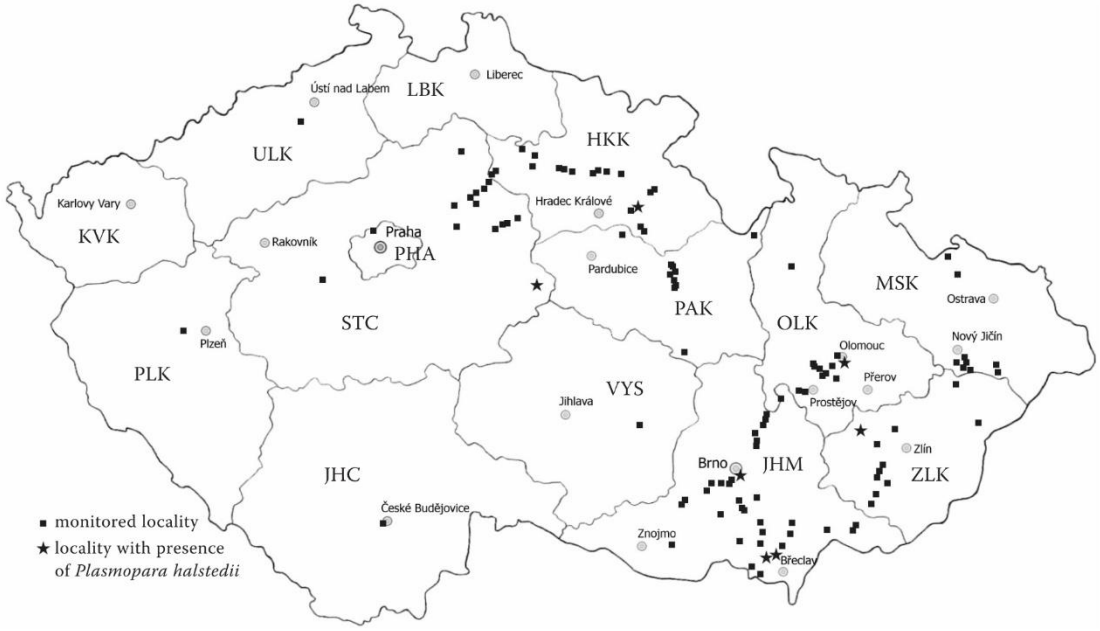


Figure 1. Map of sunflower localities monitored in the Czech Republic during 2007–2012 (squares) with records of *Plasmopara halstedii* infections (marked by asterisks)

Regions in the Czech Republic: MSK – Moravskoslezský Region, ZLK – Zlínský Region, OLK – Olomoucký Region, JHM – Jihomoravský Region, HKK – Královéhradecký Region, PAK – Pardubický Region, VYS – Vysočina Region, LBK – Liberecký Region, STC – Středočeský Region, PHA – Hlavní město Praha Region, JHC – Jihočeský Region, ULK – Ústecký Region, KVK – Karlovarský Region, PLK – Plzeňský Region

locality (date of observation, geographical location, type of habitat, approximate size of sunflower cultivar population/field, developmental stage of plants, and sunflower genotype/hybrid if known) (Table 1). Disease intensity (DI) in the population of sunflower cultivars was estimated using 0–4 scale, i.e. 0 – no plants with visible symptoms of disease in the host population; 1 – only plants with secondary infection or individual plants with systemic infection (less than 5% of plants in the host population); 2 – ca. 5–25% plants in the host population with systemic infection; 3 – ca. 25–50% systemically infected plants in the host population; 4 – more than 50% of the host population shows symptoms of systemic infection. In gardens and small fields the whole crop was surveyed for *P. halstedii* occurrence, in large fields the crop was surveyed by random transects across the field, including its margins.

RESULTS

The health status of 228 populations of sunflower cultivars grown at various types of habitats (fields, gardens, front gardens, road and field margins and others) was monitored altogether in 128 localities all over the Czech Republic in 2007–2012 (Table 1). All surveyed localities and highlighted localities with confirmed *P. halstedii* records during 2007–2012 are summarised in Figure 1. The majority of the populations of sunflower cultivars was observed in gardens (135) and in fields (47) (for details see Table 2). Most of the sunflower habitats were localised in South Moravia (eastern part of the Czech Republic) and in the eastern and central part of Bohemia (Figure 1). All these areas of the Czech Republic are mostly lowland territories with advanced agriculture and rather warm climate conditions suitable for sunflower growing.

During the six-year period (2007–2012) the presence of *P. halstedii* was confirmed at seven localities (i.e. Olomouc-Holice, Brno-Chrlice, Lednice, Podivín, Kroměříž, Čáslav, and Ledce), where host plants (*H. annuus*) with downy mildew systemic infection, mostly with the pathogen sporulation, were found (Table 1). Plants with secondary infection symptoms were reported from Olomouc every year and from Brno-Chrlice and Lednice only in 2010. Repeated occurrence

Table 1. List of localities in the Czech Republic surveyed for *Plasmopara halstedii* and assessed for disease severity in 2007–2012

Locality name	No. of cultivar populations	Habitat	Cultivar population/ field size	Occurrence of *P. h.*	DI in particular year					
					2007	2008	2009	2010	2011	2012
Moravskoslezský Region (MSK)										
Frenštát pod Radhoštěm	1	G	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Hodslavice	1	G	12 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Mořkov	1	G	8 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Nový Jičín-Kojetín	2	G	20 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Nový Jičín-Žilina	1	G	5 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Štítina	1	G	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Trojanovice	1	G	8 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Životice u Nového Jičína	1	G	2 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Zlínský Region (ZLK)										
Babice	1	RM	4 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Bílovice	1	G	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Huslenky	2	G	20 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Huslenky	4	FM	15 plants	–	n.r.	0	0	0	0	n.r.
Kroměříž	**2**	**EF**	**0.2 ha**	**+**	**n.r.**	**1**	**0**	**n.r.**	**n.r.**	**nr.**
Kunovice	1	G	10 plants	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Kvasice	1	G	2 plants	–	n.r.	n.r.	n.r.	n.r.	0	0
Martinice	1	F	50–60 ha	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Ostrožská Nová Ves	1	F	25 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Ostrožská Nová Ves	1	G	10 plants	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Spytihněv	1	F	25 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Valašské Meziříčí	5	G	10 plants	–	0	0	0	0	0	n.r.
Olomoucký Region (OLK)										
Bystročice	2	RM	5 plants	–	0	0	n.r.	n.r.	n.r.	n.r.
Charváty	3	G	10 plants	–	0	n.r.	0	0	n.r.	n.r.
Drahanovice	1	F	20 ha	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Drahanovice	2	G	10 plants	–	0	0	n.r.	n.r.	n.r.	n.r.
Drahany	2	G	10 plants	–	0	n.r.	0	n.r.	n.r.	n.r.
Mostkovice	2	G	4–10 plants	–	n.r.	n.r.	0	n.r.	0	n.r.
Oldřichov	1	G	1 plant	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Olomouc-Holice	**6**	**EF**	**0.5–1 ha**	**+**	**2**	**2**	**3**	**1**	**2**	**1**
Olomouc-Lazce	2	G	7 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Olomouc-Nedvězí	1	FM	10 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Olomouc-Nedvězí	1	G	50 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Olšany u Prostějova	1	G	7 plants	–	n.r.	0	n.r.	n.r.	n.r.	n.r.
Plumlov	4	G	1–15 plants	–	0	0	0	0	n.r.	n.r.
Slatinice	2	G	2–5 plants	–	0	0	n.r.	n.r.	n.r.	n.r.
Jihomoravský Region (JHM)										
Blučina	4	FG	10 plants	–	0	0	0	n.r.	0	
Brno-Chrlice	**5**	**EF**	**2 ha**	**+**	**n.r.**	**1**	**0**	**2**	**2**	**0**
Březina	1	RM	1 plant	–	n.r.	0	n.r.	n.r.	n.r.	n.r.
Čejč	2	FM	10 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Čejkovice	1	F	50 ha	–	n.r.	0	n.r.	n.r.	n.r.	0
Hajany	1	F	50 ha	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Hustopeče	1	F	50 ha	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Jedovnice	2	G	4 plants	–	0	n.r.	n.r.	n.r.	0	n.r.
Kotvrdovice	1	G	10 plants	–	n.r.	0	n.r.	n.r.	n.r.	n.r.

Table 1 to be continued

Locality name	No. of cultivar populations	Habitat	Cultivar population/ field size	Occurrence of *P. h.*	DI in particular year					
					2007	2008	2009	2010	2011	2012
Jihomoravský Region (JHM)										
Křtiny	3	G	7–10 plants	–	n.r.	0	0	0	n.r.	n.r.
Lechovice	1	F	10 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Lednice	1	FG	7 plants	–	n.r.	0	n.r.	n.r.	n.r.	n.r.
Lednice	1	F	40 ha	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Lednice	**3**	**EF**	**5 ha**	**+**	**n.r.**	**n.r.**	**n.r.**	**3**	**1**	**2**
Lipovec	3	G	5–10 plants	–	0	n.r.	0	0	n.r.	n.r.
Moravské Bránice	3	G	1–10 plants	–	0	0	n.r.	0	n.r.	n.r.
Moravský Krumlov	1	F	0.5 ha	–	n.r.	0	n.r.	n.r.	n.r.	n.r.
Moravský Krumlov	1	RM	1 plant	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Moutnice	1	F	4 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Nosislav	2	G	20–50 plants	–	0	n.r.	0	n.r.	n.r.	n.r.
Nosislav	2	FG	20 plants	–	n.r.	n.r.	n.r.	0	0	n.r.
Ochoz u Brna	3	G	2–4 plants	–	n.r.	n.r.	0	0	0	0
Ořechov	2	F	80 ha	–	n.r.	0	n.r.	n.r.	0	n.r.
Ořechov	2	G	1–10 plants	–	0	0	n.r.	n.r.	n.r.	n.r.
Ořechov	1	FM	5 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Podivín	**6**	**F**	**1–50 ha**	**+**	**0**	**0**	**2**	**3**	**2**	**0**
Pohořelice	2	F	15–30 ha	–	0	0	n.r.	n.r.	n.r.	n.r.
Ratíškovice	3	G	10 plants	–	0	n.r.	n.r.	0	0	n.r.
Rybníky	1	F	10 ha	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Sedlec	2	F	50 ha	–	0	0	n.r.	n.r.	n.r.	n.r.
Silůvky	1	F	40 ha	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Starovičky	4	F	0.2–100 ha	–	0	0	0	0	n.r.	n.r.
Starovičky	2	FG	1–5 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Starovičky	1	G	2 plants	–	n.r.	n.r.	n.r.	n.r.	0	n.r.
Strážnice	1	FG	15 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Valtice	1	FG	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Velké Bílovice	1	FG	4 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Velké Němčice	2	FM	20–30 plants	–	n.r.	0	0	n.r.	n.r.	n.r.
Velké Němčice	4	F	15–250 ha	–	n.r.	0	0	0	0	n.r.
Věstonice	1	F	50–60 ha	–	n.r.	0	n.r.	n.r.	n.r.	n.r.
Vnorovy	3	FM	30–40 plants	–	n.r.	n.r.	0	0	0	n.r.
Zaječí	1	F	40 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Želešice	1	G	10 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Královéhradecký Region (HKK)										
Albrechtice nad Orlicí	2	G	6–10 plants	–	n.r.	n.r.	0	0	n.r.	n.r.
Dětenice	1	G	4 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Dobruška	1	G	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Holovousy	3	G	5–10 plants	–	0	n.r.	0	n.r.	0	n.r.
Hořice	2	G	10 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Jaroměř	1	G	20 plants	–	n.r.	n.r.	n.r.	0	n.r.	0
Jičín-Robousy	2	G	5 plants	–	0	n.r.	n.r.	0	n.r.	n.r.
Ledce	**3**	**G**	**4–15 plants**	**+**	**n.r.**	**n.r.**	**1**	**0**	**0**	**nr.**
Nemyčeves	1	FG	10 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Ohaveč	1	G	1 plant	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Opočno	4	G	5–10 plants	–	0	n.r.	0	0	0	0
Ostroměř	2	G	5–10 plants	–	n.r.	n.r.	n.r.	0	0	n.r.

Table 1 to be continued

Locality name	No. of cultivar populations	Habitat	Cultivar population/ field size	Occurrence of *P. h.*	DI in particular year					
					2007	2008	2009	2010	2011	2012
Třebechovice pod Orebem	1	G	2 plants	–	n.r.	n.r.	0	n.r.	n.r.	n.r.
Velichovky	2	G	3–5 plants	–	n.r.	n.r.	0	0	n.r.	n.r.
Velký Vřešťov	2	G	3–4 plants	–	n.r.	n.r.	0	0	n.r.	n.r.
Vilantice	2	G	2–10 plants	–	n.r.	n.r.	0	0	n.r.	n.r.
Žďár nad Orlicí	1	G	10 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Pardubický Region (PAK)										
Bohuňovice	1	G	5 plants	–	n.r.	0	n.r.	n.r.	n.r.	0
Chvojenec	1	F	15 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
České Heřmanice	3	G	6–10 plants	–	n.r.	0	0	n.r.	0	0
Horky	1	G	10 plants	–	n.r.	n.r.	n.r.	n.r.	0	0
Kosořín	2	G	4 plants	–	0	n.r.	0	n.r.	n.r.	n.r.
Králíky	1	G	7 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Nedvězí	1	G	50 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Sedliště	3	G	10 plants	–	0	0	n.r.	n.r.	0	0
Vysočina Region (VYS)										
Velké Meziříčí	3	G	5 plants	–	n.r.	n.r.	0	0	0	n.r.
Středočeský Region (STC)										
Beroun	1	G	5 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Choťánky	1	F	20 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Čáslav	**1**	**EF**	**0.5 ha**	**+**	**n.r.**	**n.r.**	**1**	**n.r.**	**n.r.**	**nr.**
Dlouhopolsko	1	F	20 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Kosmonosy	1	F	25 ha	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Krchleby	1	G	3 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Loučeň	3	G	5–10 plants	–	n.r.	0	0	0	n.r.	n.r.
Mcely	4	G	2–10 plants	–	0	n.r.	0	0	0	0
Mcely	1	F	20 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Milovice	1	G	10 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Odřepsy	1	F	10 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Prodašice	2	F	20 ha	–	0	n.r.	n.r.	0	n.r.	n.r.
Seletice	1	G	10 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Velenka	1	F	20 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Vlkov pod Oškobrhem	1	F	15 ha	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Zavadilka	1	G	2 plants	–	n.r.	n.r.	n.r.	0	n.r.	n.r.
Hlavní město Praha Region (PHA)										
Praha-Suchdol	4	G	10 plants	–	0	0	0	0	n.r.	n.r.
Jihočeský Region (JHC)										
České Budějovice	1	G	5 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Ústecký Region (ULK)										
Bořislav	1	G	15 plants	–	0	n.r.	n.r.	n.r.	n.r.	n.r.
Plzeňský Region (PLK)										
Nýřany	3	F	15–40 ha	–	0	0	0	n.r.	n.r.	n.r.

P. h. – *Plasmopara halstedii*; DI – degree of infection (disease severity) of downy mildew in the population of sunflower cultivars (for details see Material and Methods); Habitats: EF – experimental field, F – field, FM – field margin, FG – front garden, G – garden, RM – road margin; Records: + present (in bold), – not present, n.r. – not recorded; DI – degree intensity: 0 – no plants; 1 – less than 5% of plants; 2 – ca. 5–25% plants with systemic infection; 3 – ca. 25–50% systemically infected plants; 4 – more than 50% with symptoms of systemic infection

Table 2. Incidence of *Plasmopara halstedii* in the individual sunflower habitats

Habitat	Number of records/Number of infections by *P. halstedii*						
	2007	2008	2009	2010	2011	2012	total
Experimental field	**1[a]/1[b]**	**3/3**	**4/2**	**3/3**	**3/3**	**3/2**	**17/14**
Field	8/0	10/0	**8/1**	**15/1**	**6/1**	1/0	**48/3**
Field margin	1/0	2/0	5/0	3/0	2/0	0/0	13/0
Front garden	5/0	2/0	3/0	2/0	2/0	0/0	14/0
Garden	38/0	15/0	**25/1**	37/0	20/0	0/0	**135/1**
Road margin	1/0	2/0	1/0	0/0	1/0	0/0	5/0
Total	54/1	34/3	46/4	60/3	34/4	4/2	

[a]number of sunflower cultivar populations surveyed; [b]number of sunflower cultivar populations with detected infection; in bold – presence of infection

of the systemic disease symptoms and pathogen reproduction was confirmed at four localities, i.e. Olomouc-Holice, Brno-Chrlice, Lednice and Podivín. In the experimental field of the Central Institute for Supervising and Testing in Agriculture in Brno-Chrlice the absence of *P. halstedii* was recorded in 2009 and 2012, likely due to a shift of experimental fields used for sunflower testing and thus leaving the source of the pathogen inoculum in soil. It is evident that most records (i.e. five) of sunflower downy mildew come from the experimental fields (Olomouc-Holice, Brno-Chrlice, Lednice, Kroměříž, Čáslav) with purposedly grown susceptible cultivars where the resistance of newly selected sunflower varieties is tested on soil with the long-lasting contamination of pathogen oospores. Only two localities, a garden in Ledce and small fields with heavy and waterlogged soil in Podivín, can be supposed to be a natural occurrence of *P. halstedii*. Probably the main reason for these records is sunflower growing inconsistently with the recommended agronomical practice. In a six-year summary, the frequency of *P. halstedii* incidence was the highest in experimental fields (87%) and very low in commercial fields (4%) and in private gardens (1%).

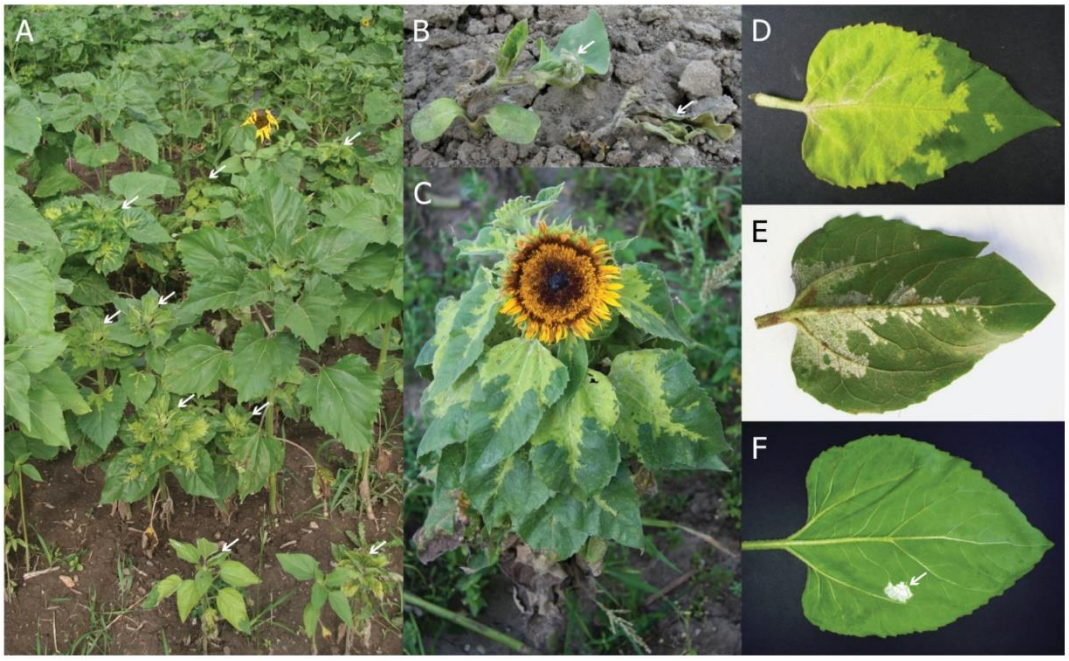


Figure 2. Symptoms of sunflower downy mildew include primary infections with A – plant dwarfing upon systemic infection, B – damping off young plants, C – failure of flowering, D – chlorosis on the adaxial side, and E – sporulation on the abaxial side of a leaf; or secondary infections characterised by F – local lesions on leaves bounded by veins

Disease severity expressed as degree of infection (DI) assessed in populations of sunflower cultivars on sites with the presence of *P. halstedii* ranged from 1 to 3 (on a 0–4 scale). Sunflower fields in Čáslav, Kroměříž, and Ledce were infected weakly (DI = 1), the other populations of sunflower cultivars were mostly infected moderately: in Podivín (DI = 2 in 2009 and 2011, or 3 in 2010), Lednice (DI = 1 in 2011 and 2012, or 3 in 2010), Brno-Chrlice (DI = 0 in 2009, 1 in 2008, or 2 in 2010-11), and Olomouc-Holice (DI = 1 in 2010 and 2012, 2 in 2007–2008 and 2011, or 3 in 2009) (for details see Table 1). Changes in the degree of infection may be attributed to variation of microclimate conditions, the level of soil contamination with pathogen oospores and genotypes of host plants. However, the variety composition of sunflower fields remained unknown in most of the localities. The observed symptoms of downy mildew primary infection included plant dwarfism (Figure 2A), seedling damping off (Figure 2B), retarded flowering (Figure 2C), deformations of the leaf blade combined with chlorotic lesions along veins on the adaxial side (Figure 2D) and frequent sporulation on the abaxial (downy) side of leaves (Figure 2E). Lesions on leaves bounded by veins (Figure 2F) indicated secondary infection. Altogether, 57 field samples of the pathogen sporulating on host plant leaves and/or stems were collected in 2007–2012 and will be used for detection of pathogen races.

DISCUSSION

According to data published by EPPO the downy mildew caused by *P. halstedii* has recently been present in the following European countries: Albania, Austria, Bulgaria, Czech Republic, Estonia, France, Germany, Greece (reported by Thanassoulopoulos & Mappas 1992), Hungary, Italy, Moldova, Romania, Slovakia, Spain, Switzerland, Turkey, Russia, and Ukraine. Within North America it is distributed in Canada, United States of America, and within South America in Argentina, Brazil, Chile, Dominican Republic, Paraguay, and Uruguay. Within Asia the pathogen was detected in China, Georgia, India, Iran, Iraq, Israel, Japan, Kazakhstan, and Pakistan. In the African continent *P. halstedii* was reported from Egypt, Ethiopia, Morocco, Kenya, and South Africa (reported by Viljoen *et al.* 1997), Uganda, and Zimbabwe.

It is evident that the global pathogenic variability of *P. halstedii* has been increasing (Gulya 2007). New hybrid cultivars evoke selection pressure which results in the emergence of new physiological races (e.g. in France, Germany, and Italy) with different virulence patterns (Sakr 2009; Virányi & Spring 2011). Phytosanitary practices are undertaken to prevent or at least reduce their spread to other countries. However, continuous and systematic monitoring of the pathogen occurrence is necessary to take appropriate preventive steps.

Our recent study has brought the first comprehensive data on *P. halstedii* incidence in the Czech Republic. It was confirmed to occur only at separate locations and mostly in sunflower experimental fields, like those of the Central Institute for Supervising and Testing in Agriculture (Brno-Chrlice, Čáslav, Lednice), Agricultural Research Institute in Kroměříž (Kroměříž) and Palacký University in Olomouc (Olomouc-Holice). Soils in these locations might be proposedly infested in the past or accidental incidence of *P. halstedii* was maintained to test new sunflower cultivars for resistance against *P. halstedii* and other diseases. Unfortunately, the source of soil contamination remains unknown. Any incidence of *P. halstedii* was detected in commercial fields unless recommended agronomical practice was omitted (i.e. in Podivín where sunflower is grown yearly on heavy and water-soaked soil). The occurrence of primary infections in commercial or hobby fields probably comes from infected sunflower seed that established *P. halstedii* in the locality some time ago. Repeated planting of seed susceptible to *P. halstedii* or inefficient fungicides might contribute to disease incidence. Wind-borne infections are not probable as *P. halstedii* is still confined to solitary and remote localities in the CR. Monitoring of harmful organisms by State Phytosanitary Administration (SPA) of the Czech Republic detected several localities mostly in South Moravia and East Bohemia (State Phytosanitary Administration 2012). Unfortunately, the records of sunflower downy mildew by SPA do not contain any information on the cultivar composition. We faced a similar task during our research and only limited information on cultivar sets was determined during the survey. Nevertheless, *P. halstedii* recorded in experimental fields of the Central Institute for Supervising and Testing in Agriculture revealed *H. annuus* cultivars lacking genes of downy mildew resistance (Labud, Oxana, Pilar and PR63A82) and cv. NK Brio bearing resistance to *P. halstedii* races 100, 703, 710 (Anonymous 2005, 2011).

The most frequent incidence of *P. halstedii* found in South Moravia in our survey is in agreement with historical records of Bojňanský (1956, 1957). It is evident from SPA annual reports that *P. halstedii* occurs scarcely also at a few more localities in the Czech Republic probably not included herein. According to our knowledge and presented results it can be assumed that preventative measures against sunflower downy mildew are effective in case that the pathogen occurrence has not been found on an epidemic scale in the Czech Republic. The detailed research focused on pathogenic variability in Czech populations of *P. halstedii* is in progress.

Acknowledgements. The authors thank Prof. Otmar Spring (Universität Hohenheim, Germany) and Prof. Ferenc Virányi (Szent István University, Gödöllő, Hungary) for critical reading of the manuscript and valuable comments. Isolates used in experiments are involved in the UPOC collection supported by the Czech National Program of Genetic Resources of Microorganisms

References

Anonymous (2005): Nově registrované odrůdy. Central Institute for Supervising and Testing in Agriculture, Brno. Available at http://www.ukzuz.cz/Uploads/7885-7-slunecnice_05pdf.aspx (accessed 12.7.2012).

Anonymous (2011): Významné hospodářské vlastnosti hybridů slunečnice roční. Central Institute for Supervising and Testing in Agriculture, Brno. Available at http://www.osevauni.cz/osiva/pdf/slunecnice_rocni_vlastnosti.pdf (accessed 12.7.2012).

Bojňanský V. (1956): Peronospora slnečnicová (*Plasmopara halstedii*) Farlow (Berl. & de Toni) v ČSR. Poľnohospodárstvo, **3**: 397–401.

Bojňanský V. (1957): Bude peronospora slunečnicová i u nás vážnou chorobou? Za Vysokou Úrodu, **6**: 19.

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.

Döken T.M. (1989): *Plasmopara halstedii* (Farl.) Berl. et de Toni in sunflower seeds and the role of infected seeds in producing plants with systemic symptoms. Journal of Phytopathology, **124**: 23–26.

Fialová P., Čech L. (2011): Slunečnice roční. Zpráva č. 10 oblastního odboru Tábor o výskytu škodlivých organismů a poruch za období od 30.5.–5.6.2011. State Phytosanitary Administration, Prague. Available at http://eagri.cz/public/web/file/121539/situacka_c_10_2011b.pdf (accessed 12.7.2012).

Gulya T.J. (2007): Distribution of *Plasmopara halstedii* races from sunflower around the world. In: Lebeda A., Spencer-Phillips P.T.N. (eds): Advances in Downy Mildew Research, Vol. 3. Proceedings 2nd International Downy Mildews Symposium. Palacký University in Olomouc and JOLA, Kostelec na Hané: 121–134.

Heiser C.B. (2008):. The sunflower (*Helianthus annuus*) in Mexico: Further evidence for a North American domestication. Genetic Resources and Crop Evolution, **55**: 9–13.

Kokeš P., Müller J. (2004): Checklist of downy mildews, rusts and smuts of Moravia and Silesia. Czech Mycology, **56**: 121–148.

Kudlíková I., Veverka K. (1999): Výsledky šlechtění slunečnice na odolnost vůči chorobám. In: Nové poznatky o vztahu hostitel-pathogen (Book of Abstracts). Czech Phytopathological Society, Prague: 37–41.

Lentz D.L., Pohl M.D., Alvarado J.L., Tarighat S., Bye R. (2008): Sunflower (*Helianthus annuus* L.) as a pre-Columbian domesticate in Mexico. Proceedings of the National Academy of Sciences of the United States of America, **105**: 6232–6237.

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**: 736–740.

Rozynek B., Spring O. (2000): Pathotypes of sunflower downy mildew in southern parts of Germany. Helia, **23**: 27–34.

Sackston W.E. (1981): Downy mildew of sunflower. In: Spencer D.M. (ed.): The Downy Mildews. Academic Press, London: 545–575.

Sakr N. (2009): Variation in aggressiveness of *Plasmopara halstedii* (sunflower downy mildew). Journal of Plant Diseases and Plant Protection, **116**: 247–251.

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**: 19–24.

Spring O. (2009): Transition of secondary to systemic infection of sunflower with *Plasmopara halstedii* – An underestimated factor in the epidemiology of the pathogen. Fungal Ecology, **2**: 75–80.

State Phytosanitary Administration (2012): Souhrnný přehled o výskytu škodlivých organismů a poruch. State Phytosanitary Administration, Prague. Available at http://eagri.cz/public/web/srs/portal/skodlive-organismy/sourhnne-prehledy-so/ (accessed 12.7.2012).

Šindelková M., Brnický P., Svobodová I., Šamánek J. (2008): Plíseň slunečnice *Plasmopara* halstedii. Ministry

of Agriculture of the Czech Republic and State Phytosanitary Administration, Prague. Available at http://eagri.cz/public/web/file/59919/Plasmopara_halstedii.pdf (accessed 12.7.2012).

Tesařová R., Juroch J., Beránek J. (2010a): Slunečnice. Zpráva o výskytu škodlivých organismů a poruch č. 15 za období 12.7.–25.7. 2010. State Phytosanitary Administration, Prague. Available at: http://eagri.cz/public/web/file/72424/_15_2010_12._7.___25._7.pdf (accessed 12.7.2012).

Tesařová R, Kroutil P, Juroch J., Beránek J. (2010b): Slunečnice. Zpráva o výskytu škodlivých organismů a poruch č. 10 za období 31.5.–6.6. 2010. State Phytosanitary Administration, Prague. Available at http://eagri.cz/public/web/file/60582/_10_2010_31._5.___6._6.pdf (accessed 12.7.2012).

Thanassoulopoulos C.C., Mappas C.B. (1992): First report of downy mildew of sunflower in Greece. Plant Disease, **76**: 539.

Tosi L., Beccari G. (2007): A new race, 704, of *Plasmopara helianthi* pathogen of sunflower downy mildew in Italy. Plant Disease, **91**: 463.

Veverka K., Křížková-Kudlíková I. (2006): Problematika testování odolnosti hybridů slunečnice vůči houbovým chorobám na provokačním pozemku. In: XVI[Ith] Czech and Slovak Conference on Plant Protection (Book of Abstracts). Czech Agricultural University, Prague: 287–288.

Viljoen A., van Wyk P.S., Nowell D.C., Gulya T.J. (1997): Occurrence of downy mildew on sunflower in South Africa. Plant Disease, **81**: 111.

Virányi F. (2002): The sunflower-*Plasmopara halstedii* pathosystem: natural and artificially induced coevolution. In: Spencer-Philips P.T.N, Gisi U., Lebeda A. (eds): Advances in Downy Mildew Research. Vol. 1. Kluwer Academic Publisher, London: 167–172.

Virányi F., Gulya T.J. (1995): Inter-isolate variation for virulence in *Plasmopara halstedii* (sunflower downy mildew) from Hungary. Plant Pathology, **44**: 619–624.

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

Received for publication August 7, 2012
Accepted after corrections August 29, 2012

Corresponding author:

Prof. Ing. Aleš Lebeda, DrSc., Univerzita Palackého v Olomouci, Přírodovědecká fakulta, Katedra botaniky, Šlechtitelů 11, 783 71 Olomouc, Česká Republika; E-mail: ales.lebeda@upol.cz

4.3. Pathogenic variability of *Plasmopara halstedii*

4.3.1. Drábková Trojanová, Z., Sedlářová, M., Pospíchalová, R., Lebeda, A. (2017): Pathogenic variability of *Plasmopara halstedii* infecting sunflower in the Czech Republic. Plant Pathology, doi: 10.1111/ppa.12722

Plant Pathology (2017) Doi: 10.1111/ppa.12722

Pathogenic variability of *Plasmopara halstedii* infecting sunflower in the Czech Republic

Z. Drábková Trojanová, M. Sedlářová, R. Pospíchalová and A. Lebeda*

Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 27, Olomouc 783 71, Czech Republic

Plasmopara halstedii was isolated from diseased sunflowers collected from eight locations in the Czech Republic from 2007 to 2014. Races of the pathogen were determined based on 84 isolates collected during the study. In total, eight races of *P. halstedii* were detected using a set of nine sunflower differential lines. Races 700, 704, 705, 710, 714 and 715 were proven by soil drench inoculation, and two additional races (730 and 770) proposed by the previously applied leaf disc inoculation method. Race 700 was the most dominant in the Czech *P. halstedii* populations, with race 710 being the second most frequent. Races 704 and 714 were found over three seasons, while other races were recorded only in one growing season (race 730 in 2010, and the new races 705 and 715 in 2014). A comprehensive study was further conducted for isolates collected in 2013–14 using an extended differential set consisting of 15 sunflower lines. According to the latter methodology which marks races with five-digit virulence codes, races 70060, 70471, 70571, 71060, 71461 and 71571 were recorded. The growing complexity of *P. halstedii* pathogenicity exhibited by the ability to infect higher numbers of differential genotypes and resulting in determination of the new pathogen races (virulence profiles) 70571, 71461 and 71571 is alarming. Although the limited number of isolates studied cannot characterize the entire pathogen diversity in the Czech Republic, the trend towards more diverse virulence in *P. halstedii* populations is clearly demonstrated by the new records of races 704, 705, 714 and 715, all capable of overcoming the resistance gene Pl_6.

Keywords: *Helianthus annuus*, *Plasmopara halstedii*, quarantine disease, races, resistance breeding, virulence variation

Introduction

Plasmopara halstedii, the causal agent of sunflower downy mildew, significantly affects yields of sunflower (Virányi & Spring, 2011). The community of oomycete molecular geneticists ranked *P. halstedii* as the 16th most important oomycete from both an economic and scientific point of view (Kamoun *et al.*, 2015). The pathogen is distributed worldwide in sunflower-growing countries (Gulya, 2007), with the exception of New Zealand and Australia where only other closely related species from the genus *Plasmopara* have been recorded on wild species from the Asteraceae family (Constantinescu & Thines, 2010; McTaggart *et al.*, 2015). *Plasmopara halstedii* is homothallic with a low level of heterozygosity (Sharma *et al.*, 2015), which goes hand in hand with multiple generations of asexual reproduction and inbreeding (Spring, 2000). Despite this, numerous races differing in virulence exist within the species (Virányi & Spring, 2011; Virányi *et al.*, 2015).

The distribution and pathogenic variability in *P. halstedii* has been studied intensively especially in North America and Europe. It was not until 1998 that a standardized set of differentials for virulence testing and a uniform race nomenclature was available (Gulya *et al.*, 1998; Trojanová *et al.*, 2017). Moreover, laboratories worldwide may still differ in their subjective assessment of the phenotypic reaction of differential lines to *P. halstedii* infection. The current consensus for defining susceptibility/resistance is based on sporulation on cotyledons and/or on true leaves. This combined evaluation (at two developmental stages of plants) has become generally accepted in the leading *Plasmopara* laboratories worldwide. Several molecular methods have been tested as promising tools for differentiation of the pathogen's intraspecific variability. Distinction of *P. halstedii* from related *Plasmopara* spp. (Choi *et al.*, 2009) as well as differentiation of *P. halstedii* isolates originating from *Rudbeckia* (Rivera *et al.*, 2016) has been successful. No reliable molecular system is available for race identification within *P. halstedii* populations on sunflower so far. Certain *P. halstedii* pathotypes can be distinguished using polymorphism analysis and KASP markers designed for CRN or RXLR effector genes (Gascuel *et al.*, 2016), partial sequence analysis of the nuclear ITS region (Spring *et al.*, 2006) or RFLP (Borovkov & McClean, 1993). Giresse *et al.* (2007) revealed 12 EST-derived markers displaying SNPs and insertion-deletion variations among isolates that seemed to be promising. Recently, sequencing and analysis of the *P. halstedii* genome provided information for future genomic studies

*E-mail: ales.lebeda@upol.cz

(Sharma *et al.*, 2015). Despite these recent efforts, molecular markers corresponding to the race virulence patterns are not available to date (Virányi & Spring, 2011).

The biology of *P. halstedii* has been studied since the 1920s (Nishimura, 1922). Pathogenic variability was first suggested in 1940 but reported only since 1960 when it was observed in the field (Novotel'nova, 1962). Until 1981, only two pathogen races, i.e. European (race 1, triplet coded virulence formula 100) and Red River Valley (race 2, 300), were recognized (Gulya *et al.*, 1991a). Carson (1981) reported race 3 (700) from Brant, North Dakota; later Gulya & Urs (1985) detected race 4 (730) in Minnesota, and Ljubich *et al.* (1988) described race 5 (770) from greenhouse experiments in Fargo, North Dakota. Subsequently, new races with more complex virulence (6 and 7, corresponding to 310 and 330, respectively) were found in North America, Argentina, Bulgaria, Hungary and France (Gulya *et al.*, 1991a). Distribution and pathogenic variability of *P. halstedii* has been continuously monitored in the major sunflower-growing areas of America and Europe. In 2007 the global number of *P. halstedii* races reached 36 (Gulya, 2007), 24 of which were reported in North America, 20 in Europe, 10 in Africa, and 5 races in both South America and Asia. In the following years, new races were found in Europe; 721 and 731 that were discovered in Bulgaria during 2007/8 (Shindrova, 2010), race 704 (Bán *et al.*, 2014b) and 714 (Bán *et al.*, 2014a) in Hungary and race 774 in France (Gascuel *et al.*, 2015). Recently, Virányi *et al.* (2015) revised the global status and revealed dramatic changes in the *P. halstedii* population, reporting 42 races worldwide, of which 36 were reported in North and South America and 24 in Europe. Recently, new races 705 and 715, originating from the Czech Republic, have been identified (Sedlářová *et al.*, 2016). Thus, over the last 40 years of intensive international monitoring, the number of *P. halstedii* physiological races has increased to at least 44.

In Europe the most comprehensive research concerning *P. halstedii* started in France in the 1950s (Gulya, 2007; Virányi *et al.*, 2015), the decade following the first European record of the pathogen occurrence in the area of former Yugoslavia (Virányi & Spring, 2011). Over the years, 17 races have been determined in French populations (Gascuel *et al.*, 2015). Pathogen variability has also been intensively studied in Hungary (Bán *et al.*, 2014a, b), Spain (Molinero-Ruiz *et al.*, 2002), Bulgaria (Shindrova, 2010), Germany (Rozynek & Spring, 2000), Italy (Tosi & Beccari, 2007) and Russia (Ivebor & Antonova, 2006).

In the Czech Republic (former Czechoslovakia), *P. halstedii* was recorded first in the 1950s (Bojňanský, 1956). Several records of sunflower downy mildew from the last decade came from research plots or the State Phytosanitary Administration, but information concerning pathogen population structure has been lacking since the start of monitoring of *P. halstedii* on cultivated sunflower in 2007 (Sedlářová *et al.*, 2013). Herein is reported results of a comprehensive study conducted during the last 8 years and data is provided on race composition of *P. halstedii* populations at eight locations in the Czech Republic.

Materials and methods

Origin of *P. halstedii* isolates

Isolates were obtained from infected sunflowers collected from eight locations in the Czech Republic (Fig. 1) during annual collecting expeditions in 2007–14. Locations represent three habitats: experimental fields with pathogen oospores present in soil (checked by laboratory growing of susceptible sunflowers in soil samples) related to research institutes and universities (Brno – Chrlice, Čáslav, Kroměříž, Lednice, Olomouc – Holice); commercial and noncommercial fields (Podivín); and gardens with ornamental sunflowers (Ledce, Lednice – Mendel University (MU)). Seven locations have been previously reported (Sedlářová *et al.*, 2013), while the eighth location was discovered in 2013. The sample from Lednice – MU was represented by a single sunflower plant with symptoms grown in an ornamental garden of Mendel University in Lednice, South Moravia, about 3 km away from Lednice.

Isolates were annually collected from field-grown sunflowers showing symptoms of systemic infection. Bulk isolates represented by detached infected leaves from single plants were collected from May to July and if possible, several bulk isolates were collected from different plants at the same location (field). Fresh zoosporangia were used for inoculation of a universal susceptible sunflower cultivar (*Helianthus annuus* 'Peredovik' or *H. annuus* 'Giganteus') (Gulya *et al.*, 1991b) and isolates obtained by this procedure were used for race determination. Isolates were stored at −20 or −80 °C, as part of the working UPOC collection (Trojanová *et al.*, 2017).

Pathogen inoculation and multiplication

Isolates of *P. halstedii* were maintained and propagated on susceptible sunflower cultivars (Peredovik or Giganteus) in growth chambers according to Trojanová *et al.* (2017) and inoculated by the whole seedling immersion (WSI) method (Cohen & Sackston, 1973). Fresh or frozen *P. halstedii* zoosporangia were washed off into distilled water and the suspension was adjusted to a concentration between 1×10^4 and 5×10^4 mL^{-1}. Three-day-old dehulled sunflower seedlings germinated between two layers of moistened filter paper in a plastic bag at 20 °C were immersed into inoculum, incubated in the dark for 2–5 h at 17–19 °C, and then planted into moistened perlite. Inoculated plants were grown in the growth chamber for 12–14 days in a 12/12 h photoperiod, 17 °C (night)/19 °C (day). Seedlings were then incubated at 17–19 °C, 100% relative humidity and dark for 12–16 h to induce sporulation. Fresh sporangia were used for new inoculation processes, race determination, or storage at −20 °C.

Sunflower differential set

The differential sets of sunflowers for race determination are summarized in Table 1. During the study period (2007–14) two testing methods were used. In 2007–11 the leaf disc inoculation (LDI) method was applied due to low amount of seed, but was later replaced by the more precise soil drench inoculation

Figure 1 Eight locations in the Czech Republic (marked by asterisks, two in Lednice) found during the period 2007–14 with records of *Plasmopara halstedii* infecting sunflower.

Table 1 Virulence formulae of the sunflower differential set for *Plasmopara halstedii* race determination[a] of Czech isolates originating from 2007 to 2014

Differential line		700	70060[b]	704	70471[b]	70571[b]	710	71060[b]	714	71461[b]	71471[b]	71571[b]	730[c]	770[c]
D-1	GB (HA-304)[d] (Peredovik)[e]	+	+	+	+	+	+	+	+	+	+	+	+	+
D-2	RHA-265	+	+	+	+	+	+	+	+	+	+	+	+	+
D-3	RHA-274	+	+	+	+	+	+	+	+	+	+	+	+	+
D-4	PMI-3 (DM-2)[e]	–	–	–	–	–	+	+	+	+	+	+	+	+
D-5	PM-17	–	–	–	–	–	–	–	–	–	–	–	+	+
D-6	803-1	–	–	–	–	–	–	–	–	–	–	–	–	+
D-7	HAR-4	–	–	–	–	+	–	–	–	–	–	+	–	–
D-8	QHP-2 (QHP-1)[d] (HAR-5)[e]	–	–	–	–	–	–	–	–	–	–	–	–	–
D-9	HA-335	–	–	+	+	+	–	–	+	+	+	+	–	–
D-10	Y7Q	0	–	0	+	+	0	–	0	–	+	+	0	0
D-11	PSC8	0	+	0	+	+	0	+	0	+	+	+	0	0
D-12	XA	0	+	0	+	+	0	+	0	+	+	+	0	0
D-13	PSS2RM	0	–	0	+	+	0	–	0	+	+	+	0	0
D-14	VAQ	0	–	0	–	–	0	–	0	–	–	–	0	0
D-15	RHA-419	0	–	0	–	–	0	–	0	–	–	–	0	0

Reaction phenotype: +, virulent; –, avirulent; 0, not tested.
[a]Modified according to Gulya *et al.* (1998), Tourvieille de Labrouhe *et al.* (2012) and Gascuel *et al.* (2015); for details on methodology see Trojanová *et al.* (2017).
[b]Five-digit codes were used for isolates originating from 2013–14.
[c]Race determined only by LDI method.
[d]Differential line used with SDI in 2012.
[e]Differential line used with LDI in 2007–11.

method (SDI). In the period 2007–11, the differential set reported by Gulya *et al.* (1998) was used with substitution in D-1 (differential line 1) of HA-821 with Peredovik. The differential set adapted from Tourvieille de Labrouhe *et al.* (2000) with HA-304 in D-1 position, PMI-3 in D-4, and QHP1 in D-8 was used during 2012, combined with SDI. Moreover, in 2012, the isolates available from previous tests were re-evaluated. From 2013, the extended set with 15 differentials, i.e. five-digit coding system, was used in combination with SDI. Based on the differential set of Tourvieille de Labrouhe *et al.* (2012), D-1 and D-8 were updated by GB and QHP2, respectively. In cases of ambiguous results on D-1, D-4 and D-8, the tests were complemented by differential lines from older differential sets.

Race determination

Races of *P. halstedii* isolates were determined according to a semiquantitative evaluation of host–pathogen reaction represented by *P. halstedii* isolates and sunflower differential lines (Trojanová *et al.*, 2017). Races were denominated by either a three- (Tourvieille de Labrouhe *et al.*, 2000) or a five-digit coding system (Tourvieille de Labrouhe *et al.*, 2012) and confirmed by at least two individual repetitions with unambiguous results. Two different methods of host–pathogen interaction evaluation were used in this study. Detailed methodology for *P. halstedii* and evaluation of tests was recently reviewed by Trojanová *et al.* (2017).

Leaf disc inoculation (LDI)

The method according to Sackston & Vimard (1988) was used for isolates collected from 2007 to 2011. Although LDI is not recommended for virulence phenotype testing as it produces false positive results (Spring *et al.*, 1997), it was applied due to low amounts of seed of sunflower differentials available for testing. Plants of *H. annuus* 'Peredovik' and nine differential lines (Table 1) were grown in moistened perlite enriched with a standard NPK fertilizer for 10–14 days in the greenhouse, until cotyledons developed large enough to cut off discs of 12 mm in diameter (using a cork borer). Leaf discs, 15 per line, were immersed into inoculum, i.e. suspension of fresh sporangia (2×10^4 mL^{-1}), incubated at 16–18 °C in the dark for 3 h, and finally laid abaxial side down to moistened filter paper. Inoculated discs were cultivated in growth chambers with a 12/12 h photoperiod, 16 °C (night)/18 °C (day) and 100% air humidity. Pathogen sporulation intensity on every leaf disc was monitored using a semiquantitative 0 to 4 scale, 5–11 days after inoculation, and total degree of infection (DI) was determined for each differential and for virulence phenotype determined (Oros & Virányi, 1987; Trojanová *et al.*, 2017). The reaction of a particular differential line was then determined as either resistant or susceptible, based on the formula:

$$P = 100 \times (\Sigma n \times v)/(x \times N)$$

where P = the total degree of infection, n = the number of leaf discs or seedlings in each assessed category, v = the degree of infection (0–3 for seedlings or 0–4 for leaf discs), x = scale range (3 for seedlings or 4 for leaf discs) and N = total number of assessed plants or leaf discs (i.e. usually 15 leaf discs or 10 seedlings). For total DI on leaf discs lower than 25% (and a sporulation intensity classed only in category 1), the differential line was classified as resistant. For DI higher than 50%, the differential line was considered susceptible. For DI 25–50%, the test was repeated. If an ambiguous reaction (DI = 25–60%) occurred repeatedly, the isolate was considered a mixture of races (e.g. isolate K11/08).

Soil drench inoculation (SDI)

The method of Goossen & Sackston (1968) was used to identify races for isolates collected from 2012 to 2014. Seeds of *H. annuus* 'Giganteus' and 9 (Tourvieille de Labrouhe *et al.*, 2000) or 15 (Tourvieille de Labrouhe *et al.*, 2012) differential lines (Table 1) were germinated for 2–3 days. At least 10 dehulled seedlings per line were planted into a tray with moistened perlite. Each plant was inoculated with approx. 10 000 fresh zoosporangia in a droplet of inoculum of known concentration (for details see Trojanová *et al.*, 2017). Inoculated seedlings were cultivated in a growing chamber with a 12/12 h photoperiod, 19 °C (day)/17 °C (night), with the first 12–16 h following inoculation in the dark. The 12th to 14th day post-inoculation, conditions of 100% relative humidity and dark were combined for 12–16 h to induce sporulation. Total DI was determined for each differential line according to a semi-quantitative 0 to 3 scale (for details see Trojanová *et al.*, 2017). For DI lower than 30% (represented again only by seedlings in category 1), the differential line was considered as resistant to *P. halstedii*. For DI higher than 50%, the differential line was considered susceptible. In ambiguous reactions (30–50%), the fresh zoosporangia from a differential line of indistinct virulence phenotype served as source of inoculum for a new test. Resistance reaction was proven as described previously by Trojanová *et al.* (2017).

Results

Eighty-four isolates of *P. halstedii* were tested, originating from eight locations in the Czech Republic collected during 2007–14 (Fig. 1). These were locations from the region of Central Moravia (Kroměříž, Olomouc – Holice), South Moravia (Brno – Chrlice, Lednice, Lednice – Mendel University (MU), Podivín) and East Bohemia (Čáslav, Ledce) (Table 2). From the locations in Čáslav, Kroměříž, Ledce and Lednice – MU, the pathogen was isolated only once during the study, whilst samples were obtained repeatedly from the other four locations (Table 3).

Because two different methods of inoculation were used (LDI, SDI) and both three- and five-digit *P. halstedii* race coding systems were applied (Table 1), only the first three digits are mentioned in summarizing results (e.g. race 71060 is equal to 710 but not vice versa, as the reaction on differentials from the last two triplets might differ). Use of LDI (until 2011) and SDI (after 2012) was distinguished. When possible, older isolates with race determination by LDI were also re-evaluated by SDI. Because race 700 was determined consistently by both methods (Table 4), the validity of the LDI test for race 700 was considered. However, LDI could provide false positive results (Table 4), e.g. race 710 resulting from SDI in isolate P9/10, as well as in isolates H24/11 and H25/11 was previously determined as 730 and 770, respectively (Table 4). Although the limits of LDI were apparent, isolates of races 730 (B3/10) and 770 (P6/19, B1/10, B5/10, B6/10 and L17/11) could not be retested. Therefore, these results cannot be confirmed or disproved.

In total, eight races were determined during the study, but only six races (700, 704, 705, 710, 714 and 715) were confirmed by the SDI method. The most abundant race in the Czech Republic was race 700, detected in each location, except Lednice – MU. However, it is obvious that race 700, being frequent in *P. halstedii* populations during 2007–11, has been replaced in part by more virulent races. The second most abundant race found in this study was 710, detected since 2010 in pathogen populations at locations with a continuous presence of *P. halstedii*, esp. Olomouc – Holice and Podivín (Table 3). The presence of other races was transient and random. At five locations, two or more pathogen races were detected over the period 2007–14. The highest pathogenic variability of *P. halstedii* was found in Podivín; except for race 730, all other seven races present in the Czech Republic were detected there (Table 3).

Discussion

This is the first comprehensive study of *P. halstedii* pathogenic variability in the Czech Republic. However, methods of virulence testing in *P. halstedii* underwent substantial changes during this time (Trojanová *et al.*, 2017). Although LDI is fast, requires low amounts of

Table 2 *Plasmopara halstedii* isolates collected in the Czech Republic during 2007–14 with virulence formulae (VF) and methods used for race determination

Year of collection	Locality	Isolate	Inoculation method[a]	VF
2007	Olomouc – Holice	H1/07	LDI	700
		H2/07	LDI	700
2008	Brno – Chrlice	B3/08	LDI	700
		B4/08	LDI	700
		B5/08	LDI	700
		B6/08	LDI	700
	Kroměříž	K10/08	LDI	700
		K11/08	LDI	770[b]
		K12/08	LDI	700
	Olomouc – Holice	H1/08	LDI	700
		H2/08	LDI	700
2009	Čáslav	C7/09	LDI	700
		C8/09	LDI	700
	Ledce	L9/09	LDI	700
	Podivín	P1/09	LDI, SDI	700
		P2/09	LDI	700
		P3/09	LDI	700
		P4/09	LDI	700
		P5/09	LDI	700
		P6/09	LDI	770
2010	Brno – Chrlice	B1/10	LDI	770
		B2/10	LDI	700
		B3/10	LDI	730
		B4/10	LDI	700
		B5/10	LDI	770
		B6/10	LDI	770
	Lednice	L12/10	LDI	700
		L13/10	LDI	700
		L14/10	LDI	700
	Podivín	P7/10	LDI	700
		P8/10	LDI	700
		P9/10	LDI, SDI	(730[c]), 710
		P10/10	LDI	700
		P11/10	LDI	700
2011	Brno – Chrlice	B2/11	LDI	700
		B3/11	LDI	700
		B4/11	LDI	700
		B5/11	LDI	700
		B6/11	LDI	704
		B8/11	LDI, SDI	700
		B10/11	LDI	700
	Lednice	L16/11	LDI	700
		L17/11	LDI	770
		L18/11	LDI	700
		L19/11	LDI	700
	Olomouc – Holice	H22/11	LDI	700
		H23/11	LDI, SDI	700
		H24/11	LDI, SDI	(770[c]), 710
		H25/11	LDI, SDI	(770[c]), 710
	Podivín	P11/11	LDI, SDI	714
		P12/11	LDI, SDI	(704[c]), 714
		P15/11	LDI, SDI	704
2012	Lednice	L10/12	SDI	704
	Olomouc – Holice	H1/12	SDI	710
		H2/12	SDI	710
		H3/12	SDI	710
		H4/12	SDI	710
		H5/12	SDI	710
		H7/12	SDI	710
		H8/12	SDI	710
2013	Brno – Chrlice	B8/13	SDI	71060
		B9/13	SDI	71060
		B13/13	SDI	71060
	Lednice	L1/13	SDI	70471
		L3/13	SDI	704
		L4/13	SDI	700
		L6/13	SDI	70471
	Lednice – MU[d]	M7/13	SDI	71461
	Olomouc – Holice	H15/13	SDI	71060
		H16/13	SDI	71060
		H17/13	SDI	71060
		H18/13	SDI	71060
		H19/13	SDI	71060
2014	Olomouc – Holice	H12/14	SDI	71060
		H13/14	SDI	71060
		H16/14	SDI	71060
		H18/14	SDI	70060
		H19/14	SDI	70060
		H20/14	SDI	71060
	Podivín	P2/14	SDI	70571
		P6/14	SDI	71571
		P9/14	SDI	71060
		P10/14	SDI	71571
		P11/14	SDI	71471

[a]LDI, leaf disc immersion; SDI, soil drench inoculation.
[b]Virulence formula has been tested repeatedly with ambiguous results, i.e. the isolate appears to represent a mixture of races 700 and 770.
[c]Race determined only by LDI method.
[d]Lednice – MU (Mendel University) is *c.* 3 km from Lednice.

plant material, and is well suited for pathogen multiplication (Spring *et al.*, 1998) or metalaxyl resistance testing (Rozynek & Spring, 2001), it is not ideal for *P. halstedii* variability testing. The hypocotyl of the plant is excluded in this method; however, as this is where the putative hypersensitive reaction takes place during an incompatible reaction (Radwan *et al.*, 2005), *P. halstedii* can still produce spores even during an incompatible interaction with a resistant host (Spring *et al.*, 1997). Because of this non-standard interaction, there can be differential line misinterpretations as false susceptible. Differential lines D6 (803-1) and D9 (HA-335) (Spring *et al.*, 1997) can be especially problematic. Initially the laboratory did not possess sufficient material of sunflower differentials, thus the LDI method was used in 2007–11. The authors were aware that the use of different methods may have influenced the results in the first years of the survey. Thus the following conclusions are based primarily on the SDI method, and isolates with the virulence codes 730 and 770 resulting only from LDI testing are provided with a note. During the first 5 years of *P. halstedii* monitoring and pathogenicity testing,

Table 3 Geographical distribution of *Plasmopara halstedii* races in the Czech Republic during 2007–14

Location	2007	2008	2009	2010	2011	2012	2013	2014	No. of races
Brno – Chrlice		700		700, 730[a], 770[a]	700, 704		710		5
Čáslav			700						1
Kroměříž		700, 770[a]							2
Ledce			700						1
Lednice				700	700, 770[a]	704	704, 700		3
Lednice – MU[b]							714		1
Olomouc – Holice	700	700			700, 710	710	710	700, 710	2
Podivín			700, 770[a]	700, 710	704, 714			705, 710, 714, 715	7

[a]Race determined by LDI method.
[b]Lednice – MU (Mendel University) is *c.* 3 km from Lednice.

Table 4 Comparison of *Plasmopara halstedii* race determination using techniques of leaf disk immersion (LDI) and soil drench inoculation (SDI)

	Technique	
Isolate	LDI	SDI
P1/09	700	700
B8/11	700	700
H23/11	700	700
P15/11	704	704
P9/10	730	710
H24/11	770	710
H25/11	770	710
P11/11	714	714
P12/11	704	714

races 700, 704, 730 and 770 were detected. However, races 730 and 770 were never later proved by the SDI method in the given isolates and therefore the validity of these results remains speculative. On the other hand, the presence of races 700 and 704 were proven by the SDI method, confirming the presence of these races in the Czech Republic. Moreover, further testing revealed that both LDI and SDI give the same result for race 700. Thus, the presence of race 700 in the Czech Republic during 2007–11 is confirmed. The most diverse *P. halstedii* population, i.e. the highest number of races, was found at the seminatural (noncommercial crop, small field such as hobby garden) location in Podivín. A suitable environment in this habitat over many years fostered repeated cycles of the pathogen, resulting in the occurrence of new races 705 and 715 (Sedlářová *et al.*, 2016). It cannot be excluded that the mixed culture habitats facilitated evolution of the new races through exposure of different sunflower lines to the pathogen, compared to the monoculture crop habitats with a rather unidirectional selection pressure (Virányi *et al.*, 2015).

Even under standard conditions (precise SDI method for inoculation, new and extended sunflower differential set, cultivation of single zoospore isolates avoiding presence of various races in bulk isolates), *P. halstedii* race testing varies between researchers and remains partly subjective (Trojanová *et al.*, 2017). Furthermore, several problematic differential lines, even those replaced by alternative lines (Tourvieille de Labrouhe *et al.*, 2000, 2012), still do not generate clear results (data not shown). Problems were experienced here with D1 HA-304 which, although generally susceptible, often did not reach a DI of more than 50%, although the DI of the control line (*H. annuus* 'Giganteus') was 100%. A similar situation occurred with D4 PMI-3, although the older D4 DM-2 line showed a clear susceptible reaction. On the other hand, D8 QHP1 constantly produced a highly susceptible phenotype (sporulation intensity 3 according to semiquantitative scale for seedlings) in 1–3 plants out of 10 even in a clearly incompatible interaction (segregation for specific virulence factors during increase of that line might be the cause); the differential was later ranked as resistant. These limitations were addressed by repeating tests with inoculum isolated from these problematic lines and comparing the results with older differential lines bearing the same genetic background. This seemed to be resolved in the case of D1 (GB) and D8 (QHP2), which resulted in a clear reaction. In contrast, a substitute genotype should be considered for D4 PMI-3.

The pathogenic variability of the *P. halstedii* population has significantly increased since the1970s (Zimmer, 1974). In the Czech Republic only two races were reliably detected until 2010 (considering the fact that SDI uncovered the tested 730 and 770 to be 710). The most remarkable shift occurred in 2011 with detection of 704 and 714, and in 2012 with 710 becoming prevalent, while at least six races were present in 2014. However, the total number of detected races might have increased due to the discovery of new locations where *P. halstedii* is present (sunflowers as host plants are not grown on a location every year) and application of more precise determination by SDI since 2012. In the last years of the survey, four races, 70471, 71461, 70571 and 71571, were detected with high virulence complexity which overcome Pl_6 and even Pl_{15} genes. Beside these results, new occurrences of races 704 and 714 have been reported from Hungary (Bán *et al.*, 2014a,b) as well as a recent prevalence of race 714 in France (Virányi *et al.*, 2015).

The occurrence of *P. halstedii* and changes in its population up to 2007 seems to be minimal in Argentina,

Russia and Spain (Virányi *et al.*, 2015). However, it is unclear if lack of new races of *P. halstedii* in those countries is due to low dynamics in *P. halstedii* populations or less intensive monitoring. In the present study, *P. halstedii* screening started in 2007 and regrettably there is no data from the period prior to 2007 to compare, although the occurrence of *P. halstedii* in the Czech Republic has been known since the 1950s (Bojňanský, 1956). However, changes in *P. halstedii* virulence dynamics in the Czech Republic are more apparent even in this relatively short time period.

The high pathogenic variability in *P. halstedii* is known in many countries where various races have been recognized, e.g. 17 races in France (Gascuel *et al.*, 2015), 24 in Canada and 23 in the USA (Virányi *et al.*, 2015), and from these, usually one or several races are predominant (Gulya, 2007). In the Czech Republic, races 700 and 710 can be considered the most dominant. Race 700 has been constantly present in the population since 2007 and race 710, which first appeared in the Czech Republic in 2011, may actually have been present as early as 2008, considering that SDI revealed 730 and 770 tested by LDI to be 710. This is consistent with previous studies indicating that race 700 is the most frequent and prevalent race in Europe and is present in at least 10 European countries (Virányi *et al.*, 2015). Race 700 was also predominant in Hungary, Bulgaria and Italy (Gulya, 2007). In Germany, races 710 and 730 were the most frequent (Rozynek & Spring, 2000). In France races 700 and 703, together with 710, widespread in 1990s, prevailed in the past (Gulya, 2007), while in 2014, race 714 was dominant (Virányi *et al.*, 2015).

The phenomenon of increasing complexity of virulence formulae has been recorded in *P. halstedii* populations. For example, Rashid (1993) recorded that races 100 and 300, previously dominant in the Canadian *P. halstedii* population, were replaced by races 700 and 730 since 1988. A similar trend was also found in the present study. The results demonstrate that race 700, dominant until 2011, then became replaced by races with more complex virulence formulae (e.g. race 710) in the Czech Republic during 2007–14. It does not imply that race 700 disappeared from the population, but most probably it is still present in the bulk isolates, mixed with other more virulent races. It can be assumed that single sporangium or zoospore lines can be identified as different, either less or more virulent races than the original bulk isolate (Molinero-Ruiz *et al.*, 1998, 2002). The idea that single zoospore isolates can lead to more precise race determinations, especially in instances where multiple races are present in a field, was proven in this study. However, the method is highly time-consuming with a recovery ratio of about 2% (data not shown) and thus may not be convenient for routine tests.

The emergence of new *P. halstedii* races can be explained by several hypotheses. Intensive cultivation of sunflower, introduction of new sunflower cultivars bearing novel resistance genes (*Pl*) and/or selection pressure caused by the wide deployment of several resistance genes together should forestall the evolution of new races and may force the pathogen to adapt to new conditions (Virányi & Spring, 2011; Virányi *et al.*, 2015). Resistance gene Pl_6 (from HA-335) used in several sunflower hybrids was first overcome by race 304 in France in 2000 and since 2009 outbreaks of sunflower downy mildew in fields with Pl_6 resistant hybrids has been reported from the USA (Virányi *et al.*, 2015). The key process in generating variability within *P. halstedii* populations probably represents outcrossing and selfing events (Gascuel *et al.*, 2015). For example, recombination between isolates with pathotype profiles 703 and 704 probably led to emergence of race 707 in France (Delmotte *et al.*, 2008). Asexual genetic recombination between *P. halstedii* isolates (Spring & Zipper, 2006) in combination with mutation in clonal lineages (Delmotte *et al.*, 2008) can lead to emergence of new pathotypes. Future studies of *P. halstedii* will hopefully bring reliable molecular markers for identifying individual races so that the isolates can be distinguished from each other without time-consuming cultivation and testing. The first molecular tool, almost successful in this task, was presented by Gascuel *et al.* (2016) who succeeded in discriminating some (but not all) multi-isolate clusters belonging to the same physiologic race using 22 KASP (competitive allele specific PCR) markers designed on polymorphic effector genes involved in the host–pathogen recognition. Hopefully, even more specific molecular markers will be designed in the future to facilitate fast routine race determination.

This study brings the first insight into pathogenic variability of *P. halstedii* in the Czech Republic over the last decade. The authors are aware that a limited number of collected isolates and identified races cannot reflect the entire dynamics in pathogen populations. However, at least six different races (proved by the SDI method) found during the eight-year study, as well as the occurrence of new highly virulent races, represents valuable data for sunflower growers. In the Czech Republic sunflower is grown rather extensively, and rarely-found downy mildew does not represent a great danger to local production. However, emergence of new races, even if occurring in scattered locations, can represent a great danger to local production. New races with a high complexity of virulence can become a major threat to monocultures once they have contact with a susceptible crop. Thus even small pathogen populations can contribute to the common European *P. halstedii* genetic pool. The information that races of sunflower downy mildew with more complex virulence (705 and 715) exist in the Czech sunflower fields should be of concern.

Acknowledgements

Seed of sunflower differentials was kindly provided by Dr T. J. Gulya (USDA Fargo, ND, USA) and Professor K. Veverka (CRI, Prague, Czech Republic) in 2007. The authors are grateful to Tomáš Bartůšek, Karel Stojaspal and Klára Dobešová who participated in the race testing. The project has been financed by grants from the

Ministry of Education, Youth and Sports of the Czech Republic (MSM 6198959215), the Ministry of Agriculture of the Czech Republic (QH71254) and Palacký University in Olomouc (IGA UP PrF-2017-001). Isolates used for the experiments were part of the working collection of UPOC supported by the Czech National Programme of Genetic Resources of Microorganisms (Ministry of Agriculture of the Czech Republic). There are no potential conflicts of interest.

References

Bán R, Kovács A, Körösi K, Perczel M, Turóczi G, 2014a. First report on the occurrence of a new pathotype, 714, of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. *Plant Disease* **98**, 1580.

Bán R, Kovács A, Perczel M, Körösi K, Turóczi G, 2014b. First report on the increased distribution of pathotype 704 of *Plasmopara halstedii* in Hungary. *Plant Disease* **98**, 844.

Bojňanský V, 1956. Peronospora slnečnicová (*Plasmopara halstedii*) Farlow (Berl. & de Toni) v ČSR. [Sunflower downy mildew (Plasmopara halstedii) Farlow (Berl. & de Toni) in Czechoslovakia.]. *Pol'nohospodárstvo* **3**, 397–401.

Borovkov AY, McClean PE, 1993. A tandemly repeated sequence from the *Plasmopara halstedii* genome. *Gene* **124**, 127–30.

Carson ML, 1981. New race of *Plasmopara halstedii* virulent on resistant sunflowers in South Dakota. *Plant Diseases* **65**, 842–3.

Choi YJ, Kiss L, Vajna L, Shin HD, 2009. Characterization of *Plasmopara* species *Ambrosia artemisiifolia*, and notes on PH, based on morphology and multiple gene phylogenesis. *Mycological Research* **113**, 1127–36.

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

Constantinescu O, Thines M, 2010. *Plasmopara halstedii* is absent from Australia and New Zealand. *Polish Botanical Journal* **55**, 293–8.

Delmotte F, Giresse X, Richard-Cervera S, *et al.*, 2008. Single nucleotide polymorphisms reveal multiple introductions into France of *Plasmopara halstedii*, the plant pathogen causing sunflower downy mildew. *Infection, Genetics and Evolution* **8**, 534–50.

Gascuel Q, Martinez Y, Boniface MC, Vear F, Pichon M, Godiard L, 2015. The sunflower downy mildew pathogen *Plasmopara halstedii*. *Molecular Plant Pathology* **16**, 109–22.

Gascuel Q, Bordat A, Sallet E, *et al.*, 2016. Effector polymorphisms of the sunflower downy mildew pathogen *Plasmopara halstedii* and their use to identify pathotypes from field isolates. *PLoS ONE* **11**, e0148513.

Giresse X, Tourvieille de Labrouhe D, Richard-Cervera S, 2007. Twelve polymorphic expressed sequence tags-derived markers for *Plasmopara halstedii*, the causal agent of sunflower downy mildew. *Molecular Ecology Notes* **7**, 1363–5.

Goossen PG, Sackston WE, 1968. Transmission and biology of sunflower downy mildew. *Canadian Journal of Botany* **46**, 5–10.

Gulya TJ, 2007. Distribution of *Plasmopara halstedii* races from sunflower around the world. In: Lebeda A, Spencer-Phillips PTN, eds. *Advances in Downy Mildew Research*. Olomouc, Czech Republic: Kostelec na Hané JOLA and Palacký University, 121–34.

Gulya TJ, Urs NVRR, 1985. A new race of sunflower downy mildew. *Phytopathology* **75**, 1339.

Gulya TJ, Sackston WE, Virányi F, Masirevic S, Rashid KY, 1991a. New races of the sunflower downy mildew pathogen (*Plasmopara halstedii*) in Europe and North and South America. *Journal of Phytopathology* **132**, 303–11.

Gulya TJ, Miller JF, Virányi F, Sackston WE, 1991b. Proposed internationally standardized method for race identification of *Plasmopara halstedii*. *Helia* **14**, 11–20.

Gulya TJ, Tourvieille de Labrouhe D, Masirevic S, Penaud A, Rashid K, Virányi F, 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). In: Gulya TJ, Vear F, eds. *Proceedings of the International Sunflower Association Symposium III, Sunflower Downy Mildew*. Fargo, ND, USA: International Sunflower Association, 130–6.

Ivebor MB, Antonova TC, 2006. Population of disease causing downy mildew of sunflower in the North Caucasus. In: ???? ???? ed. *Proceedings of the International Scientific Conference on Modern Problems of Scientific Supplying of Sunflower Production*. Krasnodar, Russia: VNIIMK, 105–9.

Kamoun S, Furzer O, Jones JDG, *et al.*, 2015. The top 10 oomycete pathogens in molecular plant pathology. *Molecular Plant Pathology* **16**, 413–44.

Ljubich A, Gulya TJ, Miller JF, 1988. A new race of sunflower downy mildew in North America. *Phytopathology* **78**, 1508.

McTaggart AR, Shuey LS, McKennna SG, Davis RI, Shivas RG, 2015. *Plasmopara sphagneticolae* sp. nov. (Peronosporales) on *Sphagneticola* (Asteraceae) in Australia. *Australasian Plant Pathology* **44**, 81–5.

Molinero-Ruiz ML, Melero-Vara JM, Gulya TJ, 1998. Pathogenic characterization of *Plasmopara halstedii* isolates from Spain. In: Gulya TJ, Vear F, eds. *Proceedings of the International Sunflower Association Symposium III, Sunflower Downy Mildew*. Fargo, ND, USA: International Sunflower Association, 26–9.

Molinero-Ruiz ML, Domínguez J, Melero-Vara JM, 2002. Races of isolates of *Plasmopara halstedii* from Spain and studies on their virulence. *Plant Disease* **86**, 736–40.

Nishimura M, 1922. Studies in *Plasmopara halstedii*. *Journal of the College of Agriculture, Hokkaido Imperial University, Sapporo, Japan* **11**, 185–210.

Novotel'nova NS, 1962. *Plasmopara halstedii* as a composite species (the basis for the taxonomic division of the genus *Plasmopara* on Compositae). *Botanical Zhurnal (Moscow & Leningrad)* **47**, 970–81.

Oros G, Virányi F, 1987. Glasshouse evaluation of fungicides for the control of sunflower downy mildew (*Plasmopara halstedii*). *Annals of Applied Biology* **110**, 53–63.

Radwan O, Mouzeyar S, Venisse JS, Nicolas P, Bouzidi MF, 2005. Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a delayed hypersensitive response within the hypocotyls. *Journal of Experimental Botany* **56**, 2683–93.

Rashid KY, 1993. Incidence and virulence of *Plasmopara halstedii* on sunflower in western Canada during 1988–1991. *Canadian Journal of Plant Pathology* **15**, 206–10.

Rivera Y, Salgado-Salazar C, Gulya TJ, Crouch JA, 2016. Newly emerged populations of *Plasmopara halstedii* infecting rudbeckia exhibit unique genotypic profiles and are distinct from sunflower infecting strains. *Phytopathology* **106**, 752–61.

Rozynek B, Spring O, 2000. Pathotypes of sunflower downy mildew in southern parts of Germany. *Helia* **23**, 27–34.

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**, 309–12.

Sackston WE, Vimard B, 1988. Leaf disc immersion (LDI) inoculation of sunflower with *Plasmopara halstedii* for *in vitro* determination of host–pathogen relationships. *Plant Disease* **72**, 227–9.

Sedlářová M, Trojanová Z, Lebeda A, 2013. Distribution and harmfulness of *Plasmopara halstedii* on sunflower in the Czech Republic. *Plant Protection Science* **49**, 1–10.

Sedlářová M, Pospíchalová R, Drábková Trojanová Z, Bartůšek T, Slobodianová L, Lebeda A, 2016. First report of *Plasmopara halstedii* new races 705 and 715 on sunflower from Czech Republic – short communication. *Plant Protection Science* **52**, 182–7.

Sharma R, Xia X, Cano LM, *et al.*, 2015. Genome analyses of the sunflower pathogen *Plasmopara halstedii* provide insights into effector evolution in downy mildews and *Phytophthora*. *BMC Genomics* **16**, 741.

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**, 19–24.

Spring O, 2000. Homothallic sexual reproduction in *Plasmopara halstedii*, the downy mildew of sunflower. *Helia* **23**, 19–26.
Spring O, Zipper R, 2006. Evidence for asexual genetic recombination in sunflower downy mildew, *Plasmopara halstedii*. *Mycological Research* **110**, 657–63.
Spring O, Rozynek B, Zipper R, 1997. Leaf disk inoculation – a useful tool for selecting infections of sunflower downy mildew at low inoculum concentration, but inappropriate to pathotype characterization. *Journal of Phytopathology* **145**, 189–91.
Spring O, Rozynek B, Zipper R, 1998. Single spore infections with sunflower downy mildew. *Journal of Phytopathology* **146**, 577–9.
Spring O, Bachofer M, Thines M, Riethmüller A, Göker M, Oberwinkler F, 2006. Intraspecific relationship of *Plasmopara halstedii* isolates differing in pathogenicity and geographic origin based on ITS sequence data. *European Journal of Plant Pathology* **114**, 309–15.
Tosi L, Beccari G, 2007. A new race, 704, of *Plasmopara helianthi* pathogen of sunflower downy mildew in Italy. *Plant Disease* **91**, 493.
Tourvieille de Labrouhe D, Gulya TJ, Masirevic S, Penaud A, Rashid KY, Virányi F, 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). In: *Proceedings of the 15th International Sunflower Conference*. Toulouse, France: International Sunflower Association, I 61–6.
Tourvieille de Labrouhe D, Walser P, Jolivot D *et al.*, 2012. Proposal for improvement of sunflower downy mildew race nomenclature. In: *Proceedings of the 18th International Sunflower Conference*. Paris, France: International Sunflower Association, 322–7.
Trojanová Z, Sedlářová M, Gulya TJ, Lebeda A, 2017. Methodology of virulence screening and race characterization of *Plasmopara halstedii*, and resistance evaluation in sunflower – review. *Plant Pathology* **66**, 171–85.
Virányi F, Spring O, 2011. Advances in sunflower downy mildew research. *European Journal of Plant Pathology* **129**, 207–20.
Virányi F, Gulya TJ, Tourvieille de Labrouhe D, 2015. Recent changes in the pathogenic variability of *Plasmopara halstedii* (sunflower downy mildew) populations from different continents. *Helia* **38**, 149–62.
Zimmer DE, 1974. Physiological specialization between races of *Plasmopara halstedii* in America and Europe. *Phytopathology* **64**, 1465–7.

4.3.2. Sedlářová, M., Pospíchalová, R., Drábková Trojanová, Z., Bartůšek, 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(2), 182–187.

doi: 10.17221/7/2016-PPS

First Report of *Plasmopara halstedii* New Races 705 and 715 on Sunflower from the Czech Republic – Short Communication

Michaela SEDLÁŘOVÁ, Romana POSPÍCHALOVÁ, Zuzana DRÁBKOVÁ TROJANOVÁ, Tomáš BARTŮŠEK, Lucie SLOBODIANOVÁ and Aleš LEBEDA

Department of Botany, Faculty of Science, Palacký University in Olomouc, Olomouc, Czech Republic

Abstract

Sedlářová M., Pospíchalová R., Drábková Trojanová Z., Bartůšek 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 Protect. Sci., 52: 182–187.

Downy mildew caused by *Plasmopara halstedii* significantly reduces annual yields of sunflower. At least 42 races of *P. halstedii* have been identified around the world. For the first time to our knowledge, races 705 and 715 of *P. halstedii* have been isolated, originating from sunflower plants collected at a single site (Podivín, South-East Moravia) in the Czech Republic at the beginning of June 2014. This enlarges the global number of the so far identified and reported races of *P. halstedii* to 44. The increasing complexity of *P. halstedii* pathogenicity led to race identification newly by a five-digit code. According to this new nomenclature, the two races of *P. halstedii* recorded in the Czech Republic are characterised by virulence profiles 705 *71* and 715 *71*.

Keywords: *Helianthus annuus* L.; resistance; sunflower downy mildew; virulence formula

Biotrophic parasite *Plasmopara halstedii* (Farl.) Berl. et de Toni (1888), ranked in the kingdom Chromista, class Oomycetes, family Peronosporaceae, causes downy mildew of sunflower. The mechanisms of plant infection and pathogen reproduction were first studied already a century ago (Nishimura 1922). Systemically infected sunflowers do not produce well ripened achenes which causes high economic losses to the farmers. Moreover, oospores of *P. halstedii* can contaminate soil for several years. Thus sunflower downy mildew has been in the list of quarantine diseases in Europe since 1992, and in the Czech Republic ten years later (Spurný 2005). Global distribution of the pathogen has been reported, with the exception of Australia and New Zealand (Gulya 2007).

Sunflower downy mildew control relies mostly on resistant hybrids and fungicide-treated seed. Currently, ca. 20 downy mildew resistance genes (Pl_1–Pl_{17}, Pl_{21}, and Pl_{ARG}) have been characterised from cultivated and wild species of sunflowers (Gascuel *et al.* 2015; Qi *et al.* 2015). Their utilisation in resistance breeding accelerates microevolution of the oomycete as new races with complex virulence formulas emerge. The existence of *P. halstedii* races was confirmed in the 1970s when a new race was detected in the USA which was able to overcome sunflower resistance gene Pl_1, which ensured resistance to the European race (Zimmer 1974). Changes in virulence of *P. halstedii* in 1980s led to the introduction of a new nomenclature system based on the reaction of nine sunflower differential lines bearing various *Pl* resistance genes and race identification by a triplet code (Sackston *et al.* 1990; Gulya *et al.* 1991; Gulya 1995). Differential lines were produced by USDA (United States Department of Agriculture, USA), INRA (Institut National de la Recherche Agronomique, France), and IFVC (Institute of Field and Vegetable Crops, Novi Sad, Serbia). Until 2007,

Supported by the Internal Grant Agency of the Palacký University in Olomouc, Czech Republic, Grant No. IGA_PrF_2016_001.

doi: 10.17221/7/2016-PPS

18 races had been reported from Europe (Virányi *et al.* 2015) and 20 races from America (Gulya 2007). Over the past 7 years this number increased to a total of 24 races in Europe and 36 in America. The history of the research of *P. halstedii* pathogenic variability has been precisely reviewed by Virányi *et al.* (2015). Permanent changes in pathogen virulence have been reflected by modifications in the sunflower differential set (Tourvieille de Labrouhe *et al.* 2000, 2012). The tentatively adopted set consists of fifteen sunflower differential lines gathered in five triplets and *P. halstedii* races are designated by a five-digit code (Gascuel *et al.* 2015). Despite a long lasting discussion the six additional lines proposed by French researchers have yet to be internationally endorsed, hopefully during the upcoming International Sunflower Association meeting in Turkey (2016).

In the Czech Republic (formerly Czechoslovakia) the first records of sunflower downy mildew date to 1950s (Bojňanský 1956, 1957). However, a detailed study of the occurrence and pathogenic variability of *P. halstedii* was initiated at our department in 2007. During annual surveys we collected and gradually identified six races, i.e. 700, 704, 710, 714, 730, and 770 (Sedlářová *et al.* 2013; Trojanová *et al.* 2013).

This paper brings information on the occurrence of two new races of *P. halstedii*, 705 and 715, isolated for the first time ever from infected sunflowers in the south-eastern part of the Czech Republic.

MATERIAL AND METHODS

Disease symptoms, specimen collection, and isolate maintenance. In June 2014, during our annual field expedition to the south-east of the Czech Republic, sunflower plants with symptoms of primary infection by *Plasmopara halstedii* were recorded in one wet field near the town of Podivín, South Moravia. Plants at the stage of ca. 4 true leaves were heavily infected with pronounced sporulation of *P. halstedii*. The plants were pulled out, put separately on moisture filter paper in plastic boxes, and transferred to our lab. Eleven bulk isolates (one per plant) were obtained by washing zoosporangia off the leaves with distilled water and subjected to sub-cultivation on susceptible sunflower line, *Helianthus annuus* cv. Giganteus, lacking resistance genes to *P. halstedii*. A short-time storage of *P. halstedii* sporangia on host tissues was performed at –20°C, a long-time storage, if needed prior to pathogenicity/fungicide resistance tests, was held at –80°C. According to our experience, vitality of sporangia decreases rapidly after 2–3 months of storage at –20°C but isolates can be safely maintained up to 6 months at –80°C. Five isolates showed a novel pattern when tested on a set of differential lines of sunflower.

Inoculation procedures for pathogen race identification. To determine the race of *P. halstedii*, isolate seedlings were inoculated according to the soil drenching method (Goosen & Sackston 1968) using a set of 15 differential lines of sunflower (Gascuel *et al.* 2015). An overview of the differential set together with the present resistance genes is given in Table 1. Sunflower seeds originating from INRA, France (lines GB, QHP2, Y7Q, PSC8, XA, PSS2RM, VAQ, RHA419) and from dr. T. Gulya, USDA ARS (lines RHA-265, RHA-274, PMI-3, PM-17, 803-1, HAR-4, Ha-335) were increased by self-pollination in summer 2014 and derived seeds were used for pathotests. Seeds were disinfected by immersion in 0.7% sodium hypochlorite (15% solution of Savo□; Bochemie, Bohumín, Czech Republic) for 10 min, rinsed thoroughly by tap water, and placed on moisture filter paper in Petri dishes at room temperature and darkness for 2–4 days to germinate. Seedlings of sunflower differential lines (20 per line) with an optimal radicle length (1–2 cm), well-developed root hairs, and free from contaminating microorganisms were selected and planted into a tray with perlite in lines. *P. halstedii* isolates, multiplied on seedlings of a susceptible sunflower cv. Giganteus, either fresh or stored frozen on host leaves, were used as a source of inoculum. Zoosporangia were washed off leaves in distilled water and the density of suspension checked using Bürker counting chamber and light microscopy. An appropriate amount of fresh inoculum was pipetted directly to each plantlet to reach the number of ca. 10 000 zoosporangia. Seedlings were covered with a thin layer of perlite and cultivated for the first 12–16 h at 17°C in darkness. Subsequent plant growth proceeded at 21/17°C, 12/12 h (day/night). Whole cultivation was done in wet perlite to ensure optimal conditions for pathogen development. Two-week-old plants were sprayed with distilled water and trays were closed in dark foil for 24 h to stimulate sporulation in humid environment. Semi-quantitative assessment of the disease intensity followed the next day according to standard methods (Oros & Virányi 1987). A week later the whole procedure of water-spraying and evaluation was repeated to observe any late-developing systemic infection. For higher confidence, testing of each isolate was conducted two to three times.

doi: 10.17221/7/2016-PPS

Table 1. Sunflower differential lines with corresponding resistance genes (modified according to that proposed by Gascuel *et al.* 2015) and virulence phenotype of new races 705 *71* and 715 *71* (+ = virulent; – = avirulent)

Set of differentials	Number	Virulence value	Sunflower line	Known *Pl* (*R* genes)	Race/virulence profile	
					705 *71*	715 *71*
	D1	1	Ha-304	–	+	+
1	D2	2	RHA-265	Pl_1	+	+
	D3	4	RHA-274	Pl_2/Pl_{21}	+	+
	D4	1	PMI-3	Pl_{PMI3}	–	+
2	D5	2	PM-17	*Pl5*	–	–
	D6	4	803-1	*Pl5* +	–	–
	D7	1	HA-R4	Pl_{15}[1]/Pl_{16}[2]	+	+
3	D8	2	QHP-2	?	–	–
	D9	4	Ha-335	Pl_6	+	+
	D10	1	Y7Q	Pl_6 –	+	+
4	D11	2	PSC8	Pl_2	+	+
	D12	4	XA	Pl_4	+	+
	D13	1	PSS2RM	Pl_6/Pl_{21}	+	+
5	D14	2	VAQ	Pl_5	–	–
	D15	4	RHA-419	Pl_{ARG}	–	–

[1]Gascuel *et al.* (2014); [2]Liu *et al.* (2012)

Degree of infection and race determination. In sunflowers two types of resistance to downy mildew conferred by *Pl* genes have been recognised: type I resistance characterised by the absence of symptoms on shoots and the absence of the pathogen above the base of hypocotyls, and type II resistance characterised by weak sporulation symptoms restricted to cotyledons, and the absence of symptoms in the upper parts of plant with the pathogen never reaching true leaves (Mouzeyar *et al.* 1994). For each plant infection degree was evaluated as the area of cotyledons/leaves covered with zoosporangiophores using the scale ranging from 0 to 3 (Oros & Virányi 1987) where 0 = no sporulation, 1 = sparse sporulation, 2 = less than 50% of cotyledon/leaf area covered, and 3 = more than 50% of cotyledon/leaf area covered with zoosporangiophores. The total degree of infection (P) was calculated for each differential line and expressed as a percentage of the maximum scores according to equation:

$$P = \frac{\sum n \times v}{x \times N} \times 100$$

where: P – total degree of infection; n – number of seedlings in each infection degree; v – infection degree (0–3 for seedlings); x – scale range = 3; N – total number of assessed seedlings

Subsequently the susceptibility/resistance of an isolate was recognised (degree of infection ≥ 50% → susceptibility; ≤ 10 → resistance; 10–50% → repetition of the experiment) and corresponding race of each isolate determined according to virulence phenotype under the rules of the proposed five-digit coding system (Gascuel *et al.* 2015).

Fungicide test. A leaf disc immersion test (Rozynek & Spring 2001) was applied to assess susceptibility/resistance of all isolates to metalaxyl. In brief, leaf discs 6 mm in diameter were cut off cotyledons of susceptible *H. annuus* cv. Giganteus plantlets with a cork borer bathed in distilled water for a short time and dried. Distilled water or 10 mg/l solution of metalaxyl (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alanine methyl ester; FLUKA Metalaxyl-M, Sigma-Aldrich spol. s.r.o., Prague, Czech Republic) was pipetted into wells of Nunc 24-well multidish (Thermo Fisher Scientific, Prague, Czech Republic). Sunflower cotyledon discs were floated on the surface by adaxial side and the centre of the upper side was inoculated by pipetting 10–15 µl of suspension containing ca. 10.000 of *P. halstedii* zoosporagia (Figure 1; inoculum preparation see above). The multi-well plate was covered and placed at 18–19°C in dark for 12 h, later in day/night

doi: 10.17221/7/2016-PPS

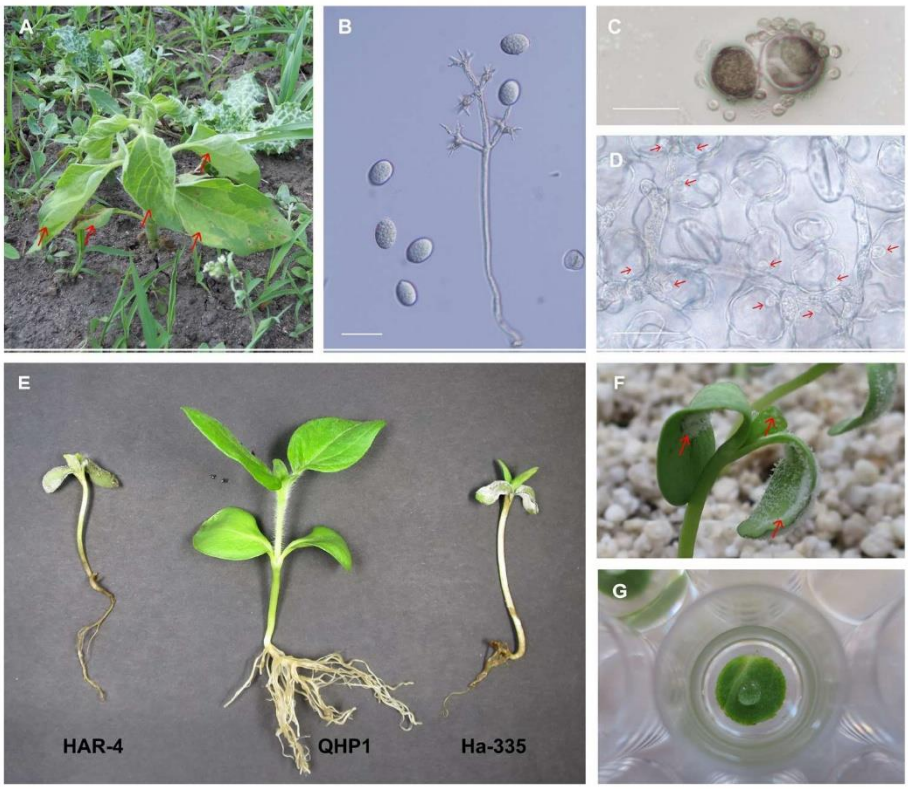


Figure 1. *Plasmopara halstedii* infected plant in the field near Podivín with typical symptoms (see arrows) of chlorotic spots and sporulation (**A**), zoosporangiophore (**B**), and zoosporangia releasing zoospores (**C**); sunflower leaf tissue with downy mildew intercellular hyphae and haustoria – marked by arrows (**D**). Reaction phenotypes of sunflower lines from the third triplet of differential set to infection by *P. halstedii* race 705 *71* (**E**) and detail of sporulation on cotyledons and first true leaves of line HAR-4 (**F**). Absence of sporulation in fungicide test (**G**). Bar in microphotographs corresponds to 50 µm (Photos (A–B) and (D–G) by M. Sedlářová and (C) Z. Drábková Trojanová)

12/12 hours. Presence of sporulation was thoroughly checked using stereomicroscope two weeks post inoculation. The presence of intensive sporulation on > 75% of inoculated control discs was the measure of a successful test.

RESULTS AND DISCUSSION

Races of *Plasmopara halstedii* isolates collected in June 2014 were specified according to a proposed nomenclature based on the virulence profile of a given isolate on 15 sunflower differential lines selected according to their resistance/susceptibility patterns (Tourvieille de Labrouhe *et al.* 2012; Gascuel *et al.* 2015). This differential set of sunflowers is specified in Table 1. The seedling inoculation pathotests repeatedly proved the presence of race 705 *71* in three, and race 715 *71* in two bulk isolates of *P. halstedii* originating from sunflower plants collected at a locality near the town of Podivín, in the south-eastern part of the Czech Republic (Figure 1). In this waterlogged field, sunflower plants with symptoms of *P. halstedii* primary infection had been repeatedly recorded in previous years (Sedlářová *et al.* 2013). New combinations of the infection phenotype of sunflower differentials which were recorded during recent testing are shown in Table 1.

Leaf disc immersion tests (Rozynek & Spring 2001) revealed susceptibility of all the tested isolates to metalaxyl, used for sunflower seed dressing. No recorded fungicide resistance is a good message for local farmers. On the other hand, they should keep agronomic practices not to sustain the pathogen life cycle and microevolution.

The survey of the *P. halstedii* research history has been nicely reviewed thanks to the cooperation of Prof. F. Virányi from Hungary, Dr. T.J. Gulya from the

doi: 10.17221/7/2016-PPS

USA, and Dr. D. Tourvieille de Labrouhe from France (Virányi *et al.* 2015). The authors summarise that until 2015 at least 42 races of *P. halstedii* have been reported, out of which 24 races came from European countries and 36 races from America. However, the virulence pattern observed in our tests has not been published yet. Several factors must be taken into account when comparing results published during the last 40 years. The increasing knowledge of *P. halstedii* intraspecific variability has been tightly linked with cultivars/genotypes of host plants used for testing. Gradual changes in the differential set of sunflower lines make the comparison of different research teams over the time rather difficult. Recently, several modifications have been made in the third triplet, e.g. at the position of D8 the USDA's line HA-R5 (Gulya *et al.* 1991) has been replaced by INRA's selection QHP-1 (Tourvieille de Labrouhe *et al.* 2000, 2012) and newly by a further reselection named QHP-2 (Pierre Desray, INRA, pers. comm.). In our tests line QHP-1 in addition to QHP-2 was used in the position of D8 and resulted in the same reaction pattern (data not shown). Other issue to be discussed is the insufficiency of information what resistance genes are present in the individual differential lines. Unfortunately, data available for several lines are confusing, e.g. Liu *et al.* (2012) mapped Pl_{16} in sunflower line HA-R4 (used at the position of D7) and Pl_{13} in HA-R5 (D8) whereas French colleagues, especially Gascuel *et al.* (2015), have recently report genes Pl_{15} in HA-R4 (D7) and Pl_1/Pl_{15} in QHP-1, a selection used instead of HA-R5 (D8). Nevertheless, such situation is not unique in plant pathology. Similar ambiguity occurs in race specific interactions where a pathogen's virulence pattern is determined on a set of host lines. Resistance genes and/or quantitative trait loci in sunflower differential lines remain to be reliably characterised by genetic methods in the future.

In the Czech Republic the occurrence of *P. halstedii* is rather limited (Sedlářová *et al.* 2013) and the incidence of the quarantine disease varies greatly depending on the weather. Detailed research of these isolated populations of *P. halstedii* is still in process but races detected so far have been similar to those reported from central European countries, especially Hungary (compare within Table 1 in Virányi *et al.* 2015). Similar to Hungarian populations of *P. halstedii*, the races 704 and 714 have recently been recorded also in the Czech Republic (Trojanová *et al.* 2013; Bán 2014a,b). To our knowledge, this paper is the first report of *P. halstedii* races 705 and 715 ever in the Czech Republic, Europe, and also worldwide. Outbreaks of *P. halstedii* races bearing new combinations of virulence overcoming resistance genes utilised in sunflower breeding are warning. However, further increasing complexity of *P. halstedii* virulence is very probable as in populations of other downy mildews infecting crops and vegetables which are intensively grown in monocultures.

References

Bán R., Kovács A., Körösi K., Perczel M., Turóczi G. (2014a): First report on the occurrence of a new pathotype, 714, of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. Plant Disease, 98: 1580–1581.

Bán R., Kovács A., Perczel M., Körösi K., Turóczi G. (2014b): First report on the increased distribution of pathotype 704 of *Plasmopara halstedii* in Hungary. Plant Disease, 98: 844.

Bojňanský V. (1956): Peronospora slnečnicová (*Plasmopara halstedii*) Farlow (Berl. & de Toni) v ČSR. Poľnohospodárstvo, 3: 397–401.

Bojňanský V. (1957): Bude peronospora slnečnicová i u nás vážnou chorobou? Za vysokou úrodu, 6: 19.

Gascuel Q., Martinez I., Boniface M.-C., Vear F., Pichon M., Godiard L. (2015): The sunflower downy mildew pathogen *Plasmopara halstedii*. Molecular Plant Pathology, 16: 109–122.

Goossen P.G., Sackston W.E. (1968): Transmission and biology of sunflower downy mildew. Canadian Journal of Botany, 46: 5–10.

Gulya T.J. (1995): Proposal of a revised system of classifying races of sunflower downy mildew. In: Proceedings 17th Sunflower Research Workshop, Jan 12–13, 1995, Fargo, USA: 76–78.

Gulya T.J. (2007): Distribution of *Plasmopara halstedii* races from sunflower around the world. In: Lebeda A., Spencer-Phillips P.T.N. (eds): Advances in Downy Mildew Research. Vol. 3. Kostelec na Hané, Palacký University in Olomouc and JOLA: 121–134.

Gulya T.J., Miller J.F., Virányi F., Sackston W.E. (1991): Proposed internationally standardized method for race identification of *Plasmopara halstedii*. Helia, 14: 11–20.

Liu Z., Gulya T.J., Seiler G.J., Vick B.A., Jan C.C. (2012): Molecular mapping of the Pl_{16} downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in sunflower. Theoretical and Applied Genetics, 125: 121–131.

Mouzeyar S., Delabrouhe D.T., Vear F. (1994): Effect of host-race combination on resistance of sunflower, *Heli-*

doi: 10.17221/7/2016-PPS

anthus annuus L., to downy mildew *Plasmopara halstedii*. Journal of Phytopathology, 141: 249–258.

Nishimura M. (1922): Studies in *Plasmopara halstedii*. Journal of College of Agriculture, Hokkaido Imperial University, XI (3): 85–210.

Oros G., Virányi F. (1987): Glasshouse evaluation of fungicides for the control of sunflower downy mildew (*Plasmopara halstedii*). Annals of Applied Biology, 110: 53–63.

Qi L.L., Long Y.M., Jan C.C., Ma G.J., Gulya T.J. (2015): Pl_{17} is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus annuus* L.). Theoretical and Applied Genetics, 128: 757–767.

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., Gulya T.J., Miller J.F. (1990): A proposed international system of designating races of *Plasmopara halstedii*. Plant Disease, 74: 721–723.

Sedlářová M., Trojanová Z., Lebeda A. (2013): Distribution and harmfulness of *Plasmopara halstedii* on sunflower in the Czech Republic. Plant Protection Science, 49: 1–10.

Spurný M. (2005): Hybridy slunečnice Rustica úspěšné v boji proti plazmopaře. In: Řepka, mák, slunečnice a hořčice, Feb 22–23, 2005, Česká zemědělská společnost na ČZU v Praze a Katedra rostlinné výroby, Prague: 164–166.

Tourvieille de Labrouhe D., Gulya T.J., Maširevic S., Penaud A., Rashid K., Virányi F. (2000): New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). In: Proceedings 5th International Sunflower Conference, June 12–15, 2012, Toulouse, France: I61–I66.

Tourvieille de Labrouhe D., Walser P., Jolivot D., Roche S., Serre F., Leguillon M., Delmotte F., Bordat A., Godiard L., Vincourt P., Vear F. (2012): Proposal for improvement of sunflower downy mildew race nomenclature. In: Proceedings 18th International Sunflower Conference, Feb 27–March 1, 2012, Mar Del Plata, Argentina: 6.

Trojanová Z., Sedlářová M., Bartůšek T., Stojaspal K., Lebeda A. (2013): Studium populace *Plasmopara halstedii* na území CR v letech 2007–2013, rozšíření a variabilita. Mykologické listy, 125: 36. (Abstract)

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

Zimmer D.E. (1974): Physiological specialization between races of *Plasmopara halstedii* in America and Europe. Phytopathology, 64: 1465–1467.

Received: 2016–01–08
Accepted after corrections: 2016–04–13
Published online: 2016–05–10

Corresponding author:

Doc. RNDr. Michaela Sedlářová, Ph.D., Univerzita Palackého v Olomouci, Přírodovědecká fakulta, Katedra botaniky, Šlechtitelů 27, 783 71 Olomouc, Česká republika; E-mail: michaela.sedlarova@upol.cz

5. DISCUSSION

5.1. Definition of *P. halstedii* taxonomic position and host range

Basic premise for study of any organism is definition of the species, in the case of pathogens complemented by determination of their host range. In these terms, *P. halstedii* is not clear-cut yet even though a great effort has been made for species concept and host range definition which underwent long-standing development (Virányi et Spring, 2011). Before the advent of molecular phylogeny, morphological features observed by accessible light or scanning electron microscopy are useful in preliminary discrimination of downy mildews (Thines, 2007) but just morphometric concept of the species often leads to description of a type later recognized as species complex (Thines et al., 2007; Voglmayr et Constantinescu, 2008; Choi et al., 2011). Moreover, detailed morphometric studies revealed variability of sporangiophores and sporangia to be influenced by their age, individual organs of the host, and current environmental factors (Hall, 1996; Runge et al., 2012). Within Peronosporomycetes dimorphism of sporangia, i.e. phenomenon known for some fungi, was recorded in white rusts (family Albuginaceae). However, these differ from downy mildews (Peronosporaceae) by mode of sporangiogenesis (Constantinescu et Thines, 2006). Some morphometric features (fine structure of (conidio)sporangiophore ultimate branchlets, ornamentation of sporangia) can be even used for phylogenetic studies (Thines, 2007) as the best combined with a plethora of genetic markers such as LSU, COX 2, β-tubulin, NADH 1 etc. (Gascuel et al., 2015). Applying morphometric together with molecular phylogeny data during revision of *Plasmopara* complex parasitic on Geraniaceae led to discovery of new species *P. praetermissa* and *P. wilsonii* (Voglmayr et al., 2006). Similarly, the revision of *Plasmopara* records newly based on morphological and molecular phylogenetic data led to reclassification of three species; i.e. *Plasmopara* parasitic on *Scorzonera* ranked under *Novotelnova scorzonerae*, *Plasmopara euphrasiae* sp. nov. segregated from *P. densa*, and *P. centaureae-mollis* relegated to synonymy of *Bremia centaureae* (Voglmayr et Constantinescu, 2008). Nowadays, combination of morphometric, molecular phylogeny and host range data seems to be the most precise way to define a pathogen species (Thines, 2014; Constantinescu et Thines, 2010).

Like other biotrophic obligate parasites, *P. halstedii* has rather narrow host range (Virányi, 2008). The host range of *P. halstedii* has not been sufficiently cleared yet and both *sensu lato* and *sensu stricto* species concepts have been presented in literature (EPPO data sheets of quarantine pests; Novoteľnova, 1962, 1970; Leppik, 1966; Gascuel et al., 2015). Though it has been described to occur on a number of Composites originally and it was also found to attack a few wild *Helianthus* species besides sunflower, little attention has been paid to alternate hosts as potential infection sources in the past. Former assumptions that *P. halstedii* affects tens of genera seems to be misleading. The first "narrowing" of *P. halstedii* host range resulted from cross-inoculation tests on a broad spectrum of potential hosts with *P. halstedii* inoculum originating from cultivated sunflowers. In result only a couple of *Helianthus* sp. (Virányi et Spring, 2011) and wild Asteraceae hosts, *Ambrosia artemisiifolia, Artemisia vulgari*s and *Xanthium strumarium,* were susceptible (Walcz et al.,

2000). *A. artemisiifolia* and *Iva xanthiifolia* may be considered *P. halstedii* wild hosts although only morphometric determination and bilateral cross-inoculation tests were successfully executed (Walcz et al., 2000; Gulya, 2002). Latest records of the natural occurrence of *P. halstedii* on wild asteraceous plant were on *Abutilon theophrasti* in Serbia (Masirevic, 2005) and *Rudbeckia fulgida* in Virginia (Hong, 2006). Host range of *P. halstedii* is therefore probably narrower than it has originally been assumed; up- to-date it includes at least 6 species in 6 genera related to *Helianthus* spp. as discriminated by cross-infectivity tests and morphologic determination of the pathogen (Virányi, 2008). Recent phylogenetic data indicate, that *sensu stricto* species concept with a narrow host range has greater support (Virányi et Spring, 2011). Revision of downy mildew samples on wild Asteraceae conserved in herbaria originally described as *P. halstedii* as well as cross-inoculation test with downy mildew isolates from non *H. annuus* hosts is a future challenge, that could shed more light in *P. halstedii* host range confusing situation.

However, the concepts of pathogens´ host range may be corrupted by host jumps, increasing the success of a pathogen strategy (Runge et al., 2017; Choi et Thines, 2015; Raffaele et al., 2010). For example, morphometry-, fatty acids- and ITS-based molecular phylogenetic analyses clearly proved that *X. strumarium* can concurrently host *P. halstedii* as well as closely related *P. angustiterminalis*, which can in turn survive on sunflower (Komjáti et al., 2007). Based on reports of Walcz et al. (2000) and Komjáti et al. (2007), there exists a possibility of encountering of *P. halstedii* from cultivated sunflower and *P. angustiterminalis* on *X. strumarium* and thus a chance for gene-flow from one pathogen species to another. Similar situation arose in *Pseudoperonospora cubensis* and *Pseudoperonospora humuli* which both can infect cultivated *Cucumis sativus* and wild host *Bryonia dioica* (Runge et Thines, 2012). In the downy mildews, an economically important group of obligate biotrophic oomycete pathogens, the transmission of inoculum between wild and culture pathosystem has been discussed for several pathogens and must be considered. Wild hosts of *P. halstedii* can serve as inoculum reservoirs providing spread of the pathogen, increase the chance for genetic exchange during hybridization (Komjáti et al., 2007) and may even selectively alter pathogen virulence (Walcz et al., 2000). Genetic and physiological diversity of sunflower downy mildew hosts within its host range and selection pressure induced by *Pl* genes incorporated in cultivated crop along with wild hosts drive evolution of pathogenic variability of *P. halstedii*. Its capacity to diverse, both in virulence and resistance fungicides, is very high, giving a continuous challenge to involved scientists.

5.2. Adequate methodology - the basis for study of *P. halstedii* pathogenic variability

Since biotrophic pathogens cannot be cultivated on (semi-)artificial media their study requires reliable methods of their isolation, multiplication, maintenance and storage on tissues of their susceptible host as well as repeatable methods of pathogenic variability and resistance screening. This requires pre-culture of host plants which makes maintenance of biotrophs challenging, time and material consuming. Manuals for cultivation and storage of some important downy mildews have been available, such as for *Hyaloperonospora*

arabidopsidis parasitic on model plant *Arabidopsis thaliana* (McDowell et al., 2011), *Bremia lactucae* (Lebeda et Petrželová, 2010) and *Pseudoperonospora cubensis* (Lebeda et Urban, 2010). Broadly accepted and reliable technique of pathogen maintenance significantly facilitates the research, influences results quality and assists at results comparison among laboratories all over the world. Information regarding sunflower downy mildew handling, intraspecific variability tests and fungicide reaction screening appeared when *P. halstedii* became a threat to sunflower production. These included methods for *in vitro* inoculation of the host tissue (Sackston et Vimard, 1988), sporangia storage (Gulya et al., 1993), pathogen multiplication (Gulya, 1996), single spore isolation and inoculation (Spring et al., 1998), fungicide resistance screening (Rozynek et Spring, 2001) or detection of *P. halstedii* oospores in soil (Gulya, 2004). In summary, previously published protocols have not covered all aspects of *P. halstedii* maintenance and handling, both in field and laboratory; moreover some of them were refuted over the time (Spring et al., 1997). So primary effort of our work was to combine known and published methods with recent experiences from both our research at the Dept. Botany and other cooperating laboratories to consolidate a generally accepted methodology for reliable *P. halstedii* handling (Trojanová et al., 2017). In summary, choosing suitable general susceptible host with no resistance genes, optimizing growth conditions and avoiding cross contamination by other pathogen isolates and/or *P. halstedii virus* are crucial features for further work with representative pathogen material.

Knowledge on intraspecific pathogenic variability of a downy mildew describing physiological races or virulence patterns is crucial for host crop resistance breeding. Methods for pathogenic variability screening of economically important downy mildews are available, such as routinely used ones for *Bremia lactucae* (Ettekoven et van Arend, 1999; Lebeda et Petrželová, 2010; Parra et al., 2016), *Pseudoperonospora cubensis* (Lebeda et Widrlechner, 2003; Lebeda et Urban, 2010). Other methods are in the stage of development, such as methodology for *Plasmopara viticola* (Gómez-Zeldón et al., 2013). All the methods mentioned above are based on reaction pattern of differential set comprising several host plants or combination of cultivars with diverse resistance genes and thus differential reactions to the pathogen. Actual differential set mirrors current knowledge of the pathogen host range and significant sources of resistance in host plants. For example, *P. cubensis* known to attack at least 60 species from family Cucurbitaceae has 12 differential lines with five different host genera, several species and cultivars of grown cucurbits (Lebeda et Urban, 2010; Lebeda et Widrlechner, 2003). However, this differential set is used only for pathotyping, not determination of races (Lebeda et al., 2013). On the contrary, *B. lactucae* has broad host range on ca 200 species in Asteraceae (Thines et al., 2010) however many isolates from wild hosts are not capable to infect cultivated lettuce and therefore 11 *formae speciales* of *B. lactuace* were established (Skidmore et Ingram, 1985; Lebeda et al., 2008). The most common wild host an source of inoculum of *B. lactucae* invading cultivated lettuce is *L. serriola* (Beharav et al., 2006) which is also broadly used in resistance breeding (Lebeda et al., 2002). Therefore differential set of *B. lactucae* consist of differentials represented only by cultivars of *L. sativa* and *L. serriola.* Recently, 28 *Dm*

genes and 23 R-factors, that provide resistance to specific isolates of *B. lactucae*, and 15 quantitative trait loci (QTLs) responsible for quantitative resistance have been known (Parra et al., 2016). Since the host range of *P. halstedii* is not defined yet precisely, sunflower downy mildew populations on close relatives are genetically distinct from those on sunflower (Rivera et al., 2016) and because *P. halstedii* is significant esp. to cultivated *H. annuus*, the differential set comprises only sunflower cultivars with diverse resistance *Pl* genes (Gulya et al., 1991a; Tourvieille de Labrouhe et al., 2012).

Sunflower differential set has been under gradual development since 1990 (Sackston et al., 1990).The first widely accepted system was based on nine differentials and race coding by three digits (Gulya, 1995) with some later modifications (Tourvieille de Labrouhe et al., 2000b). However, ambiguous reaction phenotype of some differential lines and increasing pathogenic variability of *P. halstedii* over the past 40 years induced the need for the differential set revision and extension up to recent 15 differentials and five-digit race coding (Tourvieille de Labrouhe et al., 2012; Gascuel et al., 2015). Usage of nine lines of sunflower differential set has been capable of detection of 44 *P. halstedii races* denominated with triplet codes so far (Drábková Trojanová et al., 2017). Higher number of differential lines with different genetic background leads to more precise resolution of pathogen physiological races. Our data show, that applying broaden differential set discriminates isolates previously grouped in one physiological race (Drábková Trojanová et al., 2017). Therefore revision of *P. halstedii* physiological races determined by narrower differential set is recommended for future studies.

Using differential set and semi-quantitative evaluation of disease intensity for pathogen physiological races determination is always partly subjective and dependent of the person who runs the tests. For example, Tourvieille de Labrouhe et al. (2012) proposed evaluation of weak sporulation on cotyledons as resistant reaction. Method described in this thesis (Trojanová et al., 2017) rates such type of sporulation by value 1 (limited sporulation, sporadic sporangiophores present), and calculation of total degree of infection may also result in evaluation of the differential line as resistant. This illustrates, that differential set testing method has its limits and more precise methods are needed. Attempts for screening of *P. halstedii* intraspecific variability based on genetic variability using molecular biology tools have been undertaken (Slobodianová, 2016; Chen et al., 2005; Intelmann et Spring, 2002). However, the only recently published work of Gascuel et al. (2016) based on effector polymorphism testing partly succeeded in discrimination of 10 out of 14 *P. halstedii* isolates with known physiological race based on previously testing of reaction phenotype on sunflower differential lines. Therefore the future efforts for *P. halstedii* physiological races delimitation will be probably focused on effector coding sequences in the pathogen genome.

5.3. *P. halstedii* distribution in the Czech Republic and central Europe

This thesis brings unique results on *P. halstedii* distribution in the Czech Republic based on comprehensive study conducted during past ten years at the Department of Botany, Faculty of Science, Palacký University in Olomouc (initiated in 2007 by prof. A. Lebeda and dr. M. Sedlářová). Occurrence of sunflower downy mildew in the former Czechoslovakia (mainly at the area currently belonging to Slovakia) in 1950´s (Bojňanský, 1956, 1957; Danko, 1962) ranks among the first records outside of North America. Since then only scattered notes have been available from our country (Hýsek et Veverka, 2002; Kokeš et Müller, 2004; Veverka et Křížková-Kudlíková, 2006; later Sedlářová et al., 2010, 2013). To our best knowledge the first reports of *P. halstedii* occurrence in Europe are available from Russia (Novoteľnova, 1966) and Czechoslovakia (Bojňanský, 1956) in the middle 1950´s, France in mid-1960´s (Ahmed et al., 2012), Hungary (Kurnik, 1970), former Yugoslavia and Romania (Zimmer, 1974) at the very late 1960´s, Spain in 1970´s (Molínero-Ruiz et al., 2002) and from Germany in middle 1980´s (Spring et al., 1991). Nowadays, sunflower downy mildew is distributed globally in countries producing sunflower (recently reviewed by Virányi et al., 2015), except for Australia and New Zealand, where strict phytosanitary measures are being applied (Constantinescu et Thines, 2010).

Czech Republic, till 1989 forming one state together with Slovakia, is located in the heart of Europe. Whereas the first observations of downy mildew infections on sunflower from the area of our country were done soon after its first occurrence in Russia, later records followed mainly from other communist countries that time (Hungary, Yugoslavia and Romania). Thus it could be hypothesized that *P. halstedii* might had been imported *via* contaminated sunflower seed in between Eastern European countries. On the other hand, records of *P. halstedii* occurrence among West European countries started in France in 1960´s, but others are dated to 1970´s (e.g. Spain) and 1980´s (e.g. Germany). Those times, during so called Soviet Union era, our country had rather restricted contacts with West Europe and America but an intensive exchange of material and strong influence of practices from Soviet Union. Our assumptions of *P. halstedii* origin in the area of former Czechoslovakia correspond with the hypothesis proposed by Virányi (2002) who suggested spread of *P. halstedii* from its secondary distribution centre northern of the Black Sea. It is likely that the "Iron curtain" could have affected distribution of this pathogen in Europe probably by exchange of contaminated plant material.

In the Czech Republic no data have been available concerning *P. halstedii* infections on any of six wild alternate hosts out of the genus *Helianthus* (*Xanthium strumarium, Ambrosia artemisiifolia, Artemisia vulgaris, Iva xanthifolia, Abutilon theophrasti* or *Rudbeckia fulgida*) so far. It is noteworthy to say that this is one of the reasons why our survey focused solely on cultivated sunflower, together with the need of improvement of the crop protection strategies. No records exist neither regarding *Helianthus* spp. Since the area of e.g. invasive *H. tuberosus* (Pyšek et al., 2002) and *P. halstedii* on sunflower overlap, possible host jump cannot be ruled out. Therefore monitoring of the invasive Asteraceae

should be supplemented by *P. halstedii* screening in future. The nearest populations of *P. halstedii* on alternate hosts are represented by *H. tuberosus* at Hohenheim University, Germany (prof. O. Spring, personal communication) and on *A. artemisiifolia* in southern Hungary (Walcz et al., 2000). Due to geographic isolation these do not represent high threat to Czech cultivated sunflower nor a natural source of possible recombination with *P. halstedii* in our state.

Distribution of *P. halstedii* in the Czech Republic has been limited to several local populations mainly on experimental fields of research institutions and a few incidental reports from hobby gardens. The situation is different from nearest surveyed *P. halstedii* populations in Germany where the sunflower downy mildew was reported from commercial fields (Spring et al., 1994). *P. halstedii* on experimental plots in Czech Republic is maintained in controlled conditions for sunflower field tests. However, these spots may represent a source of inoculum capable of limited spreading of the pathogen like in case of discovery an infected plant in a garden of Mendel University in Lednice, distanced ca 2,5 km from experimental field with established *P. halstedii* population. The origin of infection remains unknown; we cannot exclude neither human-assisted nor wind transmission. One "natural", and until recently well-established, population in Podivín had been probably supported by environmental factors (water-soaked soil), omitted agronomical practice and phytosanitary measures. However, the field was transformed into building parcels in 2016. Similar other "natural" reports in our country are occasional and scattered. According to CABI data (2017), *P. halstedii* is present in all countries surrounding Czech Republic, except for Poland. Limited populations of *P. halstedii* in the Czech Republic represent the northernmost boundary of the pathogen areal in central Europe but might contribute to continual distribution of this pathogen in West, South, Central and West Europe. Therefore, further monitoring to avoid the pathogen spread is highly recommended.

5.4. *P. halstedii* pathogenic variability in the Czech Republic and central Europe

After Orellana (1970) and Zimmer (1974) pointed out to physiological specialization of *P. halstedii* between races originating from America and Europe, testing of the pathogen phenotype on different sunflower genotypes has been gradually launched in the countries with high crop production and disease incidence. Intensive monitoring has been in progress esp. in USA (Gulya et al., 1991b; Gilley et al., 2016), Canada (Rashid, 1993, 2014), France (Ahmed et al., 2012), Spain (Molinero-Ruiz et al., 2002), Hungary (Virányi et Gulya, 1995; Kormány et Virányi, 1997) and Bulgaria (Shindrova, 2010). Trends and emergence of highly virulent races can be observed in local *P. halstedii* populations with the highest race variability as in France, the United States and Canada (Virányi et Spring, 2011). The global variability of *P. halstedii* based on three-digit designation of races was reviewed by Gulya (2007) and recently by Virányi et al. (2015). The pathogenic variability of *P. halstedii* population has significantly evolved since the first appearance of a new race (nominated as race 2 at that time) (Zimmer, 1974). Thus, in 2007, globally 36 races were reported (Gulya 2007), in 2012 at least 37 races were known (Liu et al., 2012), then the number increased to

42 (Virányi et al., 2015) and to date no less than 44 *P. halstedii* races are recognized (Sedlářová et al., 2016). These numbers illustrate a dramatic increase in pathogen variability since 2007 (Gulya, 2007). Information about pathogenic variability is valuable for crop producers and breeders to cultivate and develop new downy-mildew-resistant sunflower pedigrees and cultivars.

The first systematic screening of variability within sunflower downy mildew populations in the Czech Republic was initiated at our department in 2007. Initial data on *P. halstedii* physiological races in the Czech Republic come from Sedlářová et al. (2010) and Trojanová et al. (2013). Long-term monitoring over the last decade resulted in discovery of two new virulence patterns 705 and 715 (reported for the very first time by Sedlářová et al., 2016) and in the first comprehensive overview of *P. halstedii* pathogenic variability in the Czech Republic (Drábková Trojanová et al., 2017) as presented within this thesis. Intensive study of *P. halstedii* populations in the Czech Republic since 2007 led to detection of at least 6 races. As races 730 and 770 had been detected by refuted method of leaf disc immersion (LDI) pathotype testing (Spring et al., 1997) but never proved by the method of whole seeding immersion (WSI) (Gulya et al., 1991a) we decided to consider them unconvincing and thus omit them from discussion. Data presented in our paper Drábková Trojanová et al. (2017) demonstrate that the number of races in the Czech Republic increased from one in 2007 *via* four in 2011 to six in 2014. These results, i.e. trend of increasing *P. halstedii* pathogenic variability, correlate with similar observations made from France (Ahmed et al., 2012) as well as on a global scale (Virányi et al., 2015).

Pathogenic variability represents intraspecific specialisation of a pathogen raised to infect plants, in the case of biotrophs often within one host species, with different genetic backgrounds. This may be represented by a set of pathotypes, virulence patterns, physiological races or races, terminology depending on an author´s concept. In *P. halstedii* the knowledge of "physiological specialization" develops since 1970´s (Zimmer, 1974) and today embodies no less than 44 races (Sedlářová et al., 2016, Virányi et al., 2015). It is noteworthy to say, that higher intensity of sunflower growing, disease monitoring and detailed pathogen study fairly increase the number of detected races. Therefore, United States, Canada and France have been known to harbour a wide diversity of *P. halstedii* with "endemic" races descripted (Gulya, 2007). In France systematic monitoring of *P. halstedii* pathogenic variability runs at least since 1989 (Tourveielle de Labrouhe et al., 2000b), number of recognized races increased from 1 in 1966 to 14 in 2012 (Ahmed et al., 2012), with highly virulent race 714 dominant in pathogen populations (Virányi et al., 2015). In Canada the shift in *P. halstedii* population from 1xx race group to 3xx and 7xx race groups was observed in the period of 2004-2007 (Rashid, 2014). A similar situation could be followed in the Czech Republic during 2007-2014; only one race was detected in 2007, with at least 6 races found in 2014.

Worldwide the most common *P. halstedii* races have been 100 and 300 (Gulya, 2007). Though globally dominant, these two races have not been detected in the Czech Republic. One of the explanations could be that races 100 and 300, less complex in their

virulence pattern, could have been expelled by more virulent 7xx races, recently present in the Czech Republic. However, race 100 was detected in populations of *P. halstedii* in North America after 2007 even though more virulent races were present at the same area (Virányi et al., 2015). It leads us to conclusion that races 100 and 300 might have survived in population despite concurrence of more virulent races. Their absence in Czech populations was thus either not spotted due to limited number of samples in our survey (not every diseased plant could be tested) or can be explained by the fact that their phenotype of virulence was overlaid in bulk isolates during testing. To refine our assumptions, monozoosporic or at least monosporangia isolates would be required, prepared from our bulk isolates and re-tested. However, this is highly time and material consuming. Moreover, the success of monozoosporic isolation reaches ca 1-2% (Doudová, 2013), by which we lose part of the isolate possible variability. In Europe, the races mentioned above (100 and 300) complemented by races 330, 700, 710 and 730 are the most abundant ones (Virányi et al., 2015). In the Czech Republic races 700 and later 710 prevailed in *P. halstedii* populations.

Discovery of yet unique races 705 and 715 originated from our locality with the most diverse *P. halstedii* population in Podivín. However, their origin is ambiguous. It can be most probably explained by pathogen microevolution under selection pressure of partly resistant host plants in favourable environmental conditions. Applying method of multi-locus genotypes detected by EST derived markers (Ahmed et al., 2012; Giresse et al., 2007) or differentiation of pathotypes based on KASP markers (Gascuel et al., 2016) would probably explain the relationships within this diverse population and origin of 7×5 races. Despite of that, appearance of new and more virulent races is alarming and fits together with the increasing pathogenic variability of *P. halstedii* over the last decade (Virányi et al., 2015).

Races 704 and 714 detected in the Czech Republic since 2011 also appeared in other European countries. These both were recorded in France in 2002 (Ahmed et al., 2012), 704 was reported from Italy in 2006 (Tosi et Beccari, 2007) and 704 was spotted in Hungary in 2010, followed by race 714 in 2013 (Bán et al., 2014a, b). Simultaneous occurrence of both races in our country correlates with the same situation in France, where races 714, 704 and 710 were disclosed to form one cluster of multi-locus genotypes, probably evolving from race 710 introduced to France in 1988 (Ahmed et al., 2012). However, the race considered to be predominant in the Czech Republic, Hungary and Italy is the race 700 (Drábková Trojanová et al., 2017; Virányi et Gulya, 1995; Tosi et Beccari, 2007), which belongs to unrelated multi-locus genotype cluster (Ahmed et al., 2012). Race 710, considered to be the "parental" race of 704 and 714 (Ahmed et al., 2012) is absent in Italy (Virányi et al., 2015), but was found in the Czech Republic and Hungary (Drábková Trojanová et al., 2017; Virányi et Gulya, 1995). Appearance of races 704 and 714 in the Czech Republic and Hungary therefore may explained by introduction of race 710 and its consequent evolution driven by the selection pressure. This hypothesis is supported by successive presence of race 710, 704 and 714 at the same locality in Podivín. However, on other localities with records of race 704 or 714, presence of race 710 was not reliably proven, although re-testing of isolates determined primarily with LDI as 770 resulted in reclassification to 710 after WSI

pathotype tests. As our supposition is based on only limited number of isolates further isolates collection and testing are needed to be proved.

Prospective study of sunflower-*Plasmopara halstedii* pathosystem may in future bring better understanding of downy mildews phylogeny (Thines, 2014), hopefully new markers for reliable race determination or systems of pathogen-host communication *via* effectors (Gascuel et al., 2016). Last but not least up-to-date knowledge of sunflower downy mildew population variability and reaction to routinely applied fungicides helps to increase efficiency of phytosanitary measures and drives sunflower breeding towards more durable resistant *H. annuus* cultivars. Although many data on *P. halstedii* and the complex pathosystem of *H. annuus*-*P. halstedii*-*P. halstedii virus* has been enlisted, this still represents a challenging subject for future research.

6. CONCLUSIONS

This Ph.D. thesis summarizes geographical distribution and pathogenic variability of *Plasmopara halstedii* on sunflower in the Czech Republic based on eight years of monitoring.

The first part of thesis reviews methods necessary for pathogen handling, including procedures of isolation, cultivation and maintenance of *P. halstedii* isolates. It emphasizes the screening of sunflower plants based on development of the differential set and system for denomination of pathogen races. It reviews methodology for virulence screening and race determination in order to contribute to international harmonization of the methodology used in *P. halstedii* research.

Furthermore it brings original data on distribution of *P. halstedii* on cultivated sunflowers in the Czech Republic, comprising of eight localities in Central and South Moravia and Eastern Bohemia. The only locality that represents a future threat is located in Podivín, South Moravia where naturally evolving *P. halstedii* population causes sunflower downy mildew incidence repeatedly on small fields with heavy and waterlogged soil. Three other localities with settled *P. halstedii* populations and repeated incidence of sunflower downy mildew are represented by experimental plots of research institutions and, except for wind-borne infections, yet represent minimal risk for commercially grown sunflowers in the Czech Republic.

Finally, it presents the pathogenic variability of Czech *P. halstedii* populations with at least 6 physiological races. The occurrence of global distributed races 700, 710 and newer races 704, 714 was proven and for the first time ever the occurrence of races 705 and 715 were proven. Presence of two additional races, 730 and 770, spotted during the first years of the monitoring cannot be confirmed, since only method of leaf disc inoculation (LDI) was used that time, and the isolates were not maintained until to be verified with recommended soil drench inoculation (SDI) method. The change in *P. halstedii* pathogenic variability was proposed in South Moravian localities in Podivín and Lednice but limited number of samples and time of survey make it difficult for clear conclusions.

Recently the distribution *P. halstedii* in the Czech Republic is isolated. Downy mildew on cultivated sunflower is well controlled by a fungicide seed dressing and growing of certified resistant seed. However, emergence of new races with high complexity of virulence in *P. halstedii* population is alarming. Therefore, further monitoring of sunflower downy mildew and determination of race spectra is highly recommended for future.

7. SOUHRN (Summary in Czech)

Předkládaná doktorská disertační práce přináší první ucelený přehled geografického rozšíření a patogenní variability populací *Plasmopara halstedii* v České republice, založený na systematickém osmiletém studiu tohoto patogenního organismu.

První část práce se zabývá metodikou, která je pro tento typ studia nezbytná. Popisuje postupy inokulace, přemnožování a uchovávání izolátů *P. halstedii*. Práce klade důraz především na metody využívané při studiu patogenní variability tohoto organismu a na vývoj diferenciačního souboru genotypů slunečnice sloužícího k určení a označení ras. Shrnuje postup pro testování patogenní variability s cílem přispět ke sjednocení metodiky používané při studiu *P. halstedii* na mezinárodní úrovni.

Následně se práce věnuje výskytu a geografickému rozšíření *P. halstedii* v České republice. Výskyt *P. halstedii* byl zaznamenán na pěstovaných slunečnicích na 8 lokalitách na jižní a střední Moravě a ve východních Čechách. Vážnější situace byla zaznamenána pouze v Podivíně na jižní Moravě, kde populace *P. halstedii* opakovaně způsobuje plísňovitost slunečnic pěstovaných na políčkách s jílovitou a zamokřenou půdou. Zbývající lokality, kde se díky stabilní populaci *P. halstedii* plísňovitost slunečnic vyskytuje opakovaně, jsou pokusná pole výzkumných ústavů. Tyto populace *P. halstedii*, s výjimkou větrem rozšiřovaných zoosporangií, v současnosti nepředstavují pro komerčně pěstované slunečnice v České republice vážnější riziko.

Poslední část této práce přináší výsledky studia patogenní variability populace *P. halstedii* v České republice, která je reprezentována minimálně 6 fyziologickými rasami. Potvrzen byl výskyt celosvětově rozšířených ras 700, 710 a vysoce virulentních ras 704 a 714. Navíc byl popsán vůbec první výskyt nových ras 705 a 715. Výskyt dalších dvou ras 730 a 770, zaznamenaných v prvních letech studia, nebyl potvrzen. Tyto rasy byly určeny méně spolehlivou metodou inokulace listových disků (LDI) a životaschopné izoláty se bohužel nepodařilo uchovat dostatečně dlouho na to, aby mohly být výsledky potvrzeny standardní a doporučovanou metodou inokulace zasazených semenáčků (DSI). Zaznamenání více ras v posledních letech a nový výskyt ras napadajících více genotypů diferenciačního souboru slunečnice vede k možným závěrům o posunu v patogenní variabilitě v populacích *P. halstedii*. Takové závěry by však musely být ověřeny v delším časovém horizontu a na větším počtu izolátů.

Výskyt *P. halstedii* v České republice je sice lokální a plísňovitost slunečnice se v současnosti daří kontrolovat díky pěstování slunečnice z fungicidy ošetřeného osiva certifikovaných rezistentních hybridů, ale nový výskyt vysoce virulentních ras je alarmující. Pravidelný monitoring výskytu plísňovitosti u pěstovaných slunečnic a stanovení ras v objevených populacích *P. halstedii* by se měly stát rutinou, abychom i dále mohli účinně bránit většímu rozšíření této karanténní choroby u nás.

8. REFERENCES

Adl, S.M., Simpson, A.G., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., Heiss, A. (2012): The revised classification of eukaryotes. Journal of Eukaryotic Microbiology 59(5), 429-514.

Ahmed, S., Tourvieille de Labrouhe, D.T., Delmotte, D. (2012): Emerging virulence arising from hybridisation facilitated by multiple introductions of the sunflower downy mildew pathogen *Plasmopara halstedii*. Fungal Genetics and Biology 49(10), 847-855.

Albourie, J.M., Tourvieille, J., Tourvieille de Labrouhe, D. (1998): Resistance to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. European Journal of Plant Pathology 104(3), 235-242.

Bán, R., Kovács, A., Körösi, K., Perczel, M., Turóczi, G. (2014a): First report on the occurrence of a new pathotype, 714, of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. Plant Disease 98(11), 1580-1581.

Bán, R., Kovács, A., Perczel, M., Körösi, K., Turóczi, G. (2014b): First report on the increased distribution of pathotype 704 of *Plasmopara halstedii* in Hungary. Plant Disease 98(11), 844.

Bán, R., Virányi, F., Komjáti, H. (2004): Benzothiadiazole-induced resistance to *Plasmopara halstedii* (Farl.) Berl. et de Toni in sunflower. In: Spencer-Phillips, P., Jeger, M. (Eds.): Advances in Downy Mildew Research, Volume 2. Springer Netherlands, pp. 265-273.

Bartůšek, T. (2013): Patogenní variabilita *Plasmopara halstedii* a rezistence vůči metalaxylu v České republice. Bachelor thesis, Deposited at Department of Ecology, Faculty of Science, Palacký University in Olomouc, Czech Republic.

Beharav, A., Lewinsohn, D., Lebeda, A., Nevo, E. (2006): New wild *Lactuca* genetic resources with resistance against *Bremia lactucae*. Genetic Resources and Crop Evolution 53(3), 467-474.

Benz, A., Spring, O. (1995): Identification and characterization of an auxin-degrading enzyme in downy mildew infected sunflower. Physiological and Molecular Plant Pathology 46(3), 163-175.

Bojňanský, V. (1957): Bude peronospora slnečnicová i u nás vážnou chorobou? Za vysokou úrodu 6, 19.

Bojňanský, V. (1956): Peronospora slnečnicová (*Plasmopara halstedii*) Farlow (Berl. & de Toni) v ČSR. Poľnohospodárstvo 3(3), 397-401.

Borovkov, A.Y., McClean, P.E. (1993): A tandemly repeated sequence from the *Plasmopara halstedii* genome. Gene 124(1), 127–30.

Borovkova, I.G., Borovkov, A.Y., McClean, P.E., Gulya, T.J., Vick, B.A. (1992): Restriction fragment length polymorphisms and RAPD markers in DNA of *Plasmopara halstedii*, the downy mildew fungus of sunflower. In: Proceedings of 13th Sunflower International Conference. Pisa, Italy: Grafiche della Pacini, pp. 1420–1425.

CABI (2017): Datasheet: *Plasmopara halstedii* (downy mildew of sunflower). [http://www.cabi.org/isc/datasheet/41911]. Accessed 12 March 2017.

Carson, M.L. (1981): New race of *Plasmopara halstedii* virulent on resistant sunflowers in South Dakota [USA]. Plant Diseases 65(10), 842-843.

Chaki, M., Fernández-Ocaña, A.M., Valderrama, R., Carreras, A., Esteban, F.J., Luque, F, Gómez-Rodríguez, M.V., Begara-Morales, C.B., Corpas, F.C., Barroso, J.B. (2009): Involvement of reactive nitrogen and oxygen species (RNS and ROS) in sunflower–mildew interaction. Plant and Cell Physiology 50(2), 265-279.

Chalupová, J., Sedlářová, M., Helmel, M., Řehulka, P., Marchetti-Deschmann, M., Allmaier, G., Šebela, M. (2012): MALDI-based intact spore mass spectrometry of downy and powdery mildews. Journal of Mass Spectrometry 47(8), 978–986.

Chen, J., Gulya, T.J., Vick, B.A., Hu, J. (2005): TRAP analysis of molecular variability of a global collection of sunflower downy mildew, *Plasmopara halstedii*. In: Proceedings of 27th Sunflower Research Workshop.

Fargo, ND, USA: National Sunflower Association, pp. 55-62. [http://www.sunflowernsa.com/uploads/research/210/Chen_TrapAnalysis_05.pdf]. Accessed 12 March 2017.

Choi, Y.J., Thines, M. (2015): Host jumps and radiation, not co-divergence drives diversification of obligate pathogens. A case study in downy mildews and Asteraceae. PloS One 10(7), e0133655.

Choi, Y.J., Thines, M., Runge, F., Hong, S.B., Telle, S., Shin, H.D. (2011): Evidence for high degrees of specialisation, evolutionary diversity, and morphological distinctiveness in the genus *Bremia*. Fungal Biology 115(2), 102-111.

Choi, Y.J., Kiss, L., Vajna, L., Shin, H.D., (2009a): Characterization of *Plasmopara* species *Ambrosia artemisiifolia*, and notes on *Plasmopara halstedii*, based on morphology and multiple gene phylogenesis. Mycological Research 113(10), 1127–36.

Choi, Y.J., Park, M.J., Shin, H.D. (2009b): First Korean report of downy mildew on *Coreopsis lanceolata* caused by *Plasmopara halstedii*. Plant Pathology 58(6), 1171.

Constantinescu, O., Thines, M. (2010): *Plasmopara halstedii* is absent from Australia and New Zealand. Polish Botanical Journal 55(2), 293-298.

Constantinescu, O., Thines, M. (2006): Dimorphism of sporangia in Albuginales (Chromista, Peronosporomycetes). Sydowia 58(2), 178-190.

Crute, I.R., Harrison, J.M. (1988): Studies on the inheritance of resistance to metalaxyl in *Bremia lactucae* and on the stability and fitness of field isolates. Plant Pathology 37(2), 231-250.

Danko, J. (1962): Peronospóra sójová - *Peronospora manshurica* (Naumoff) Sydow - na Slovensku. Česká mykologie 16(2), 119-122.

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(5), 534-540.

Dick, M.W. (2001): Straminipilous fungi. Kluwer Academic, Dordrecht Publisher, 670 p.

Döken, M.T. (1989): *Plasmopara halstedii* (Farl.) Berl. et de Toni in sunflower seeds and the role of infected seeds in producing plants with systemic symptoms. Journal of Phytopathology 124(1), 23-26.

Doudová, T. (2013): Techniky izolace a kultivace plísně slunečnicové. Bachelor thesis, Deposited at Department of Botany, Faculty of Science, Palacký University in Olomouc, Czech Republic.

Drábková Trojanová, Z., Sedlářová, M., Pospíchalová, R., Lebeda, A. (2017): Pathogenic variability of sunflower downy mildew in the Czech Republic during 2007-2014. Plant Pathology, doi: 10.1111/ppa.12722.

eAGRI (2017): Registr přípravků na ochranu rostlin. [http://eagri.cz/public/app/eagriapp/POR/]. Accessed 20 March 2017.

EPPO (2017): Data sheets of quarantine pests: *Plasmopara halstedii*. [https://www.eppo.int/QUARANTINE/data_sheets/fungi/PLASHA_ds.pdf]. Accessed 12 March 2017.

Ettekoven, K.V., van Arend, A.J.M. (1999): Identification and denomination of "new" races of *Bremia lactucae*. In: Lebeda, A., Křístková, E. (Eds.): Eucarpia Leafy Vegetables ´99. Olomouc, Czech Republic: Palacký University in Olomouc, pp. 171-175.

Farlow, W.G. (1883): Note on some species in the third and eleventh centuries of Ellis's North American Fungi. Proceedings of the American Academy of Arts and Sciences 18, 65-85.

Fernández-Ocaña, A.M., Gómez-Rodríguez, M.V., Velasco-Negueruela, A., Camacho-Simarro, A.M., Fernández-López, C., Altarejos, J. (2004): In vivo antifungal activity of the essential oil of *Bupleurum gibraltarium* against *Plasmopara halstedii* in sunflower. Journal of Agricultural and Food Chemistry 52(21), 6414-6417.

Flor, H.H. (1955): Host–parasite interaction in flax rust – its genetics and other implications. Phytopathology 45(12), 680–685.

García-Ruiz, R., López-Rivera, S., García-Carneros, A.B., Molinero-Ruiz, L. (2012): Field incidence of sunflower downy mildew using seed dressing with metalaxyl-M. In: Proceedings of the 18th International Sunflower Conference. Mar del Plata, Argentina. [http://isasunflower.org/fileadmin/documents/aaProceedings/18thISAArgentina-vol1/Field_incidence_of_sunflower_downy_mildew_using_seed_dressing_with_metalaxyl-M.pdf]. Accessed 20 March 2017.

Gascuel, Q., Bordat, A., Sallet, E., Pouilly, N., Carrere, S., Roux, F., Vincourt, P., Godiard, L. (2016): Effector polymorphisms of the sunflower downy mildew pathogen *Plasmopara halstedii* and their use to identify pathotypes from field isolates. PloS One 11(2), e0148513.

Gascuel, Q., Martinez, Y., Boniface, M.C., Vear, F., Pichon, M., Godiard, L. (2015): The sunflower downy mildew pathogen *Plasmopara halstedii*. Molecular Plant Pathology 16(2), 109-122.

Gentzbittel, L., Mestries, E., Mouzeyar, S., Badoui, S., Vear, F., Tourvieille de Labrouhe, D., Nicolas, P. (1999): A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. Theoretical and Applied Genetics 99(1), 218-234.

Gilley, M.A., Markell, S.G., Gulya, T.J., Misar, C.G. (2016): Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers. In: Proceedings of 38th Sunflower Research Forum, Fargo, ND, USA. [https://www.sunflowernsa.com/uploads/22/plasmopara.halstedii_gilley.2016.pdf]. Accessed 12 March 2017.

Giresse, X., Tourvieille de Labrouhe, D.T., Richard-Cervera, S., Delmotte, F. (2007): Twelve polymorphic expressed sequence tags-derived markers for *Plasmopara halstedii*, the causal agent of sunflower downy mildew. Molecular Ecology Resources 7(6), 1363-1365.

Gisi, U., Cohen, Y. (1996): Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. Annual Review of Phytopathology 34(1), 549-572.

Göker, M., Voglmayr, H., Riethmüller, A., Oberwinkler, F. (2007): How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. Fungal Genetics and Biology 44(2), 105-122.

Gómez-Zeledón, J., Zipper, R., Spring, O. (2013): Assessment of phenotypic diversity of *Plasmopara viticola* on *Vitis* genotypes with different resistance. Crop Protection 54, 221-228.

Grasse, W., Spring, O. (2017): ssRNA viruses from biotrophic Oomycetes form a new phylogenetic group between Nodaviridae and Tombusviridae. Archives of Virology 162(5), 1319-1324.

Grasse, W., Spring. O. (2015): Occurrence and genetic diversity of *Plasmopara halstedii* virus in sunflower downy mildew populations of the world. Fungal biology 119(2), 170-178.

Grasse, W., Zipper, R., Totska, M., Spring, O. (2013): *Plasmopara halstedii* virus causes hypovirulence in *Plasmopara halstedii*, the downy pathogen of the sunflower. Fungal Genetics and Biology 57, 42-47.

Guchetl, S., Antonova, T., Tchelustnikova, T., Araslanova, N. (2012): Intraspecific polymorphism of *Plasmopara halstedii* affecting the sunflower in the Krasnodar region of Russian Federation revealed by molecular markers. In: Book of abstracts of the 18th International Sunflower Conference. Mar del Plata, Argentina: International Sunflower Association and Argentine Sunflower Association, pp. 79.

Gulya, T.J. (2007): Distribution of *Plasmopara halstedii* races from sunflower around the world. In: Lebeda, A., Spencer-Phillips, P.T.N. (Eds.): Advances in Downy Mildew Research. Kostelec na Hané: JOLA and Palacký University in Olomouc, Czech Republic, pp. 121-134.

Gulya, T.J. (2004): A seedling bioassay to detect the presence of *Plasmopara halstedii* in soil. In: Spencer-Phillips, P., Jeger, M. (Eds.): Advances in Downy Mildew Research, Volume 2. Springer Netherlands, pp. 233-240.

Gulya, T.J. (2002): First report of cross-infectivity of *Plasmopara halstedii* from Marshelder to Sunflower. Plant Disease 86(8), 919.

Gulya, T.J., Marty, D., Harbour, J., Holen, C., Knodel, J., Lamey, A. and Mason, P. (1999): Metalaxyl resistance in sunflower downy mildew in North America. In: Proceedings of 21st Sunflower Research Workshop. Fargo, ND, USA: National Sunflower Association, pp. 118-123.

Gulya, T., Rashid, K.Y., Masirevic, S.M. (1997): Sunflower diseases. In: Schneider, A.A. (Ed.): Sunflower Technology and Production. American Society of Agronomy, Madison, pp. 263-380.

Gulya, T.J. (1996): Everything you should know about downy mildew testing but were afraid to ask. In: Proceedings of the 18th sunflower research workshop. Fargo, ND, USA: National Sunflower Association, pp. 39-48. [http://www.sunflowernsa.com/uploads/research/639/1996_gulya_everythingdowny.pdf]. Accessed 12 March 2017.

Gulya, T.J. (1995): Proposal of a revised system of classifying races of sunflower downy mildew. In: Proceedings of 17th Sunflower Research Workshop. Fargo, ND, USA: National Sunflower Association, pp. 76-78. [http://www.sunflowernsa.com/uploads/research/686/1995_gulya_proposalrevisedsystem.pdf]. Accessed 12 March 2017.

Gulya, T.J., Masirevic, S., Thomas, C. (1993): Preservation of air-dried downy mildew sporangia in liquid nitrogen without cryoprotectants or controlled freezing. Mycological Research 97(2), 240-244.

Gulya, T.J., Freeman, T.P., Mayhew, D.E. (1992): Ultrastructure of virus-like particles in *Plasmopara halstedii*. Canadian Journal of Botany 70(2), 334-339.

Gulya, T.J., Miler, J.F., Virányi, F., Sackston, W.E. (1991a): Proposed internationally standardized method for race identification of *Plasmopara halstedii*. Helia 14, 11-20.

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

Gulya, T.J., Freeman, T.P., Mayhew, D.E. (1990): Virus-like particles in *Plasmopara halstedii*, sunflower downy mildew. Phytopathology 80, 1032.

Gulya, T.J., Urs, R. (1985): A new race of sunflower downy mildew (Abstr.). Phytopathology 75(11), 1339.

Gulya, T.J., Carson, M.L., Urs, R. (1982): Race 3 sunflower downy mildew: dristribution and sources of resistence (Abstr.). Phytopathology 72(8), 1136.

Hall, G.S. (1996). Modern approaches to species concepts in downy mildews. Plant Pathology 45(6), 1009-1026.

Hammerschmidt, R. (1999): Induced disease resistance: how do induced plants stop pathogens? Physiological and Molecular Plant Pathology 2(55), 77-84.

Heller, A., Rozynek, B., Spring, O. (1997): Cytological and physiological reasons for the latent type of infection in sunflower caused by *Plasmopara halstedii*. Journal of Phytopathology 145(10), 441-445.

Heller-Dohmen, M., Göpfert, J.C., Pfannenstiel, J., Spring, O. (2011): The nucleotide sequence and genome organization of *Plasmopara halstedii* virus. Virology Journal 8(1), 123-131.

Heller-Dohmen, M., Göpfert, J.C., Hammerschmidt, R., Spring, O. (2008): Different pathotypes of the sunflower downy mildew pathogen *Plasmopara halstedii* all contain isometric virions. Molecular Plant Pathology 9(6), 777-786.

Hong, C.X. (2006): Downy mildew of *Rudbeckia fulgida* cv. Goldstrum by *Plasmopara halstedii* in Virginia. Plant Disease 90(11), 1461.

Hu, J., Seiler, G., Kole, C. (Eds.). (2010): Genetics, genomics and breeding of sunflower. Science Publishers. Enfield, NH, USA, 335 p.

Hýsek, J., Veverka, K. (2002): *Plasmopara halstedii* na slunečnici. Úroda. [http://uroda.cz/plasmopara-halstedii-na-slunecnici]. Accessed 12 March 2017.

Intelmann, F., Spring, O. (2002): Analysis of total DNA by minisatellite and simple sequence repeat primers for the use of population studies in *Plasmopara halstedii*. Canadian Journal of Microbiology 48(6), 555-559.

Ioos, R., Laugustin, L., Rose, S., Tourvieille, J. Tourvieille de Labrouhe, D. (2007): Development of a PCR test to detect the downy mildew causal agent *Plasmopara halstedii* in sunflower seeds. Plant Pathology 56(2), 209–218.

Kamoun, S. (2007): Groovy times: filamentous pathogen effectors revealed. Current Opinion in Plant Biology 10(4), 358-365.

Kamoun, S. (2006): A catalogue of the effector secretome of plant pathogenic oomycetes. Annual Review of Phytopathology 44, 41-60.

Kokeš, P., Müller, J. (2004): Checklist of downy mildews, rusts and smuts of Moravia and Silesia. Czech Mycology 56(1/2), 121-148.

Komjáti, H., Bakonyi, J., Spring, O., Virányi, F. (2008): Isozyme analysis of *Plasmopara halstedii* using cellulose acetate gel electrophoresis. Plant Pathology 57(1), 57-63.

Komjáti, H., Walcz, I., Virányi, F., Zipper, R., Thines, M., Spring, O. (2007): Characteristics of a *Plasmopara angustiterminalis* isolate from *Xanthium strumarium*. European Journal of Plant Pathology 119(4), 421-428.

Kormány, A., Virányi, F. (1997): Studies on the virulence and aggressiveness of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium) 62, 911-918.

Körősi, K., Lázár, N., Virányi, F. (2009): Resistance to downy mildew in sunflower induced by chemical activators. Acta Phytopathologica et Entomologica Hungarica, 44(1), 1-9.

Kurnik, E. (1970): Outlook for breeding sunflowers with reference to disease resistance and improvement of oil content and quality. Proceedings of 4th International Sunflower Conference. Memphis, TN, USA.

Lebeda, A., Pavelková, J., Sedláková, B., Urban, J. (2013): Structure and temporal shift in virulence of *Pseudoperonospora cubensis* populations in Czech Republic. Plant Pathology 62(2), 336-345.

Lebeda, A., Petrželová, I. (2010): Screening for resistance to lettuce downy mildew (*Bremia lactucae*). In: Spencer, M., Lebeda, A. (Eds.): Mass Screening Techniques for Selecting Crops Resistant to Diseases, Vienna, IAEA, pp. 245-249. [http://www-pub.iaea.org/MTCD/Publications/PDF/TDL-001_web.pdf]. Accessed 12 March 2017.

Lebeda, A., Urban, J. (2010): Screening for resistance to cucurbit downy mildew (*Pseudoperonospora cubensis*). In: Spencer, M., Lebeda, A. (Eds.): Mass Screening Techniques for Selecting Crops Resistant to Diseases, Vienna, IAEA, pp. 285-294. [http://www-pub.iaea.org/MTCD/Publications/PDF/TDL-001_web.pdf]. Accessed 12 March 2017.

Lebeda, A., Sedlářová, M., Petřivalský, M., Prokopová, J. (2008): Diversity of defence mechanisms in plant-oomycete interactions: A case study of *Lactuca* spp.-*Bremia lactucae*. European Journal of Plant Pathology 122(1), 71-89.

Lebeda, A., Mazáková, J., Táborský, V. (2006): Protozoa a Chromista: taxonomie, biologie a hospodářský význam. Česká fytopatologická společnost, Praha, 92 p.

Lebeda, A., Widrlechner, M.P. (2003): A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes (Ein Testsortiment von Cucurbitaceae-Taxa für die Differenzierung der Pathotypen von *Pseudoperonospora cubensis*). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 110(4), 337-349.

Lebeda, A., Pink, D.A.C., Astley, D. (2002): Aspects of the interactions between wild *Lactuca* spp. and related genera and lettuce downy mildew (*Bremia lactucae*). In: Spencer-Phillips, P.T.N., Gisi, U., Lebeda, A. (Eds.): Advances in Downy Mildew Research. Kluwer Academic Publishers, Dordrecht, pp. 85-117.

Leppik, E.E. (1966): Origin and specialization of *Plasmopara halstedii* complex in the Compositae. FAO Plant Protection Bulletin 14, 72-76.

Liu, Z., Gulya, T.J., Seiler, G.J., Vick, B.A., Jan, C.C. (2012): Molecular mapping of the *Pl* 16 downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in sunflower. Theoretical and Applied Genetics 125(1), 121-131.

Ljubich, A., Gulya, T.J., Miller, J.F. (1988): A new race of sunflower downy mildew in North America (Abstr.). Phytopathology 78(12), 1580.

Masirevic, S. (2005): First appearance of cross-infectivity of *Plasmopara halstedii* from velvetleaf (*Abutilon theophrasti*) to sunflower. In: Abstracts of 10th Technical Consultation Meeting of the FAO-European Cooperative Research Network on Sunflower. Novi Sad, Serbia, p. 27.

Mattos, J.K.A., Pio, B.L.A., Gouveia, J., Inacio, C., Dianese, J.C. (2006): Primero relato de *Plasmopara halstedii* em *Ageratum houstonianum* (Asteraceae) no distrito federal, Brasil. Fitopatologia Brasileira 31(4), 413.

McDowell, J.M., Hoff, T., Anderson, R.G., Deegan, D. (2011): Propagation, storage, and assays with *Hyaloperonospora arabidopsidis*: a model oomycete pathogen of *Arabidopsis*. Plant Immunity: Methods and Protocols 712, 137-151.

Meliala, C., Vear, F., Tourvieille de Labrouhe, D. (2000): Relation between date of infection of sunflower downy mildew (*Plasmopara halstedii*) and symptoms development. Helia 23(32), 35-44.

Molinero-Ruiz, M.L., Dominguez, J., Gulya, T. J., Melero-Vara, J. M. (2005): Reaction of field populations of sunflower downy mildew (*Plasmopara halstedii*) to metalaxyl and mefenoxam. Helia 28(42), 65-74.

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.

Müller, U., Gisi, U. (2012): Newest aspects of nucleic acid synthesis inhibitors: Metalaxyl-M. Modern Crop Protection Compounds, Volumes 1-3, Second Edition, pp. 901-908.

Nagaraju, A., Sudisha, J., Murthy, S.M., Ito, S.I. (2012): Seed priming with *Trichoderma harzianum* isolates enhances plant growth and induces resistance against *Plasmopara halstedii*, an incitant of sunflower downy mildew disease. Australasian Plant Pathology, 41(6), 609-620.

Nandeeshkumar, P., Sudisha, J., Ramachandra, K.K., Prakash, H.S., Niranjana, S. R., Shekar, S.H. (2008a): Chitosan induced resistance to downy mildew in sunflower caused by *Plasmopara halstedii*. Physiological and Molecular Plant Pathology 72(4), 188-194.

Nandeeshkumar, P., Ramachandra Kini, K., Prakash, H.S., Niranjana, S.R., Shekar, S.H. (2008b): Induction of resistance against downy mildew on sunflower by rhizobacteria. Journal of Plant Interactions 3(4), 255-262.

Nishimura, M. (1922): Studies in *Plasmopara halstedii*. Journal of the College of Agriculture, Hokkaido Imperial University, Sapporo, Japan 11(3), 185–210.

Novoteľnova, N.S. (1970): Evroaziatskie vidy roda *Plasmopara* Schroet. na slozhnotsvetnykh. Novosti Sistematiki Nizshikh Rastenij 6, 88-92.

Novoteľnova, N.S. (1966): Downy mildew on sunflower. Izdatelstvo „Nauka“, Moskva-Leningrad. Translated from Russian, Indian National Scientific Documentation, Hillside Road New Delhi.

Novoteľnova, N.S. (1962): *Plasmopara halstedii* (Farl.) Berl. et De Toni kak sbornyj vid (Obosnovanie k taksonomicheskomu podrazdeleniyu roda *Plamopara* na slozhnotsvetnykh) / *Plasmopara halstedii* as a composite species (the basis for the taxonomic division of the genus *Plasmopara* on Compositae). Botanicheskij Zhurnal (Moscow and Leningrad) 47, 970-981.

Nunninger, C., Watson, G., Leadbitter, N., Ellgehausen, H. (1996): CGA329351: introduction of the enantiometric form of the fungicide metalaxyl. In: Proceedings of the British Crop Protection Conference – Pests and Diseases, Vol. 1. British Crop Protection Council, Farnham, pp. 41-46.

Orellana, R.G. (1970): Resistance and susceptibility to downy mildew and variability in *Plasmopara halstedii*. Bulletin of the Torrey Botanical Club 97(2), 91-97.

Oros, G., Virányi, F. (1984): Resistance of *Plasmopara halstedii* to metalaxyl in the greenhouse. Temperate Downy Mildew Newsletter 3, 22-23.

Parra, L., Maisonneuve, B., Lebeda, A., Schut, J., Christopoulou, M., Jeuken, M., McHale, L., Truco, M.J., Crute, I., Michelmore, R. (2016): Rationalization of genes for resistance to *Bremia lactucae* in lettuce. Euphytica 210(3), 309-326.

Povolný, M., Hampl, B. (2015): Přehled odrůd 2015 slunečnice (Descriptive List of Plant Varieties 2015 Sunflower). Central Institute for Supervising and Testing in Agriculture, Brno, 35 p. [http://eagri.cz/public/web/file/410302/PO_Slunecnice_2015.pdf]. Accessed 20 March 2017.

PPDB (2017a): Pesticide Properties DataBase – Metalaxyl [http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/444.htm]. Accessed 20 March 2017.

PPDB (2017b): Pesticide Properties DataBase – Metalaxyl-M [http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/445.htm]. Accessed 20 March 2017.

Pyšek, P., Sádlo, J., Mandák, B. (2002): Catalogue of alien plants of the Czech Republic. Preslia 74(2), 97-186.

Radwan, O., Mouzeyer, S., Venisse, J.S., Nicolas, P., Bouzidi, M.F. (2005): Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a delayed hypersensitive response within the hypocotyls. Journal of Experimental Botany 56(420), 2683-2693.

Raffaele, S., Farrer, R.A., Cano, L.M., Studholme, D.J., Maclean, D., Thines, M., Jiang, R.H.Y., Zody, M.C., Kunjeti, S.G., Donofrio, N.M., Meyers, B.C., Nusbaum, C., Kamoun, S. (2010): Genome evolution following host jumps in the Irish potato famine pathogen lineage. Science 330(6010), 1540-1543.

Rashid, K.Y. (2014): Changes in sunflower downy mildew races in Manitoba, 2004-2013. Poster. Agriculture and Agri-Food Canada. [http://www.sunflowernsa.com/uploads/research/1221/rashid_changes.dm.races.2004-13.poster_2014.pdf]. Accessed 12 March 2017.

Rashid, K.Y. (1993): Incidence and virulence of *Plasmopara halstedii* on sunflower in western Canada during 1988-1991. Canadian Journal of Plant Pathology 15(3), 206-210.

Ritschelová, I. et al. (2016): The statistical yearbook of the Czech Republic 2016. [https://www.czso.cz/documents/10180/48781008/320198161305.xlsx/61997569-1d0b-4644-9dc9-7560581f312f?version=1.1]. Accessed 22 March 2017.

Rivera, Y., Salgado-Salazar, C., Gulya, T.J., Crouch, J.A. (2016): Newly emerged populations of *Plasmopara halstedii* infecting rudbeckia exhibit unique genotypic profiles and are distinct from sunflower infecting strains. Phytopathology 106(7), 752-761.

Roitsch, T., Balibrea, M.E., Hofmann, M., Proels, R., Sinha, A.K. (2003): Extracellular invertase: key metabolic enzyme and PR protein. Journal of Experimental Botany 54(382), 513-524.

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(6), 309-312.

Rozynek, B., Spring, O. (2000): Pathotypes of sunflower downy mildew in southern parts of Germany. Helia 23, 27-34.

Runge, F., Gärber, U., Lebeda, A., Thines, M. (2017): Multiple host shifts from prickly lettuce give rise to Bremia lactucae on cultivated lettuce. European Journal of Plant Pathology, submitted.

Runge, F., Ndambi, B., Thines, M. (2012): Which morphological characteristics are most influenced by the host matrix in downy mildews? A case study in *Pseudoperonospora cubensis*. PloS One 7(11), e0044863.

Runge, F., Thines, M. (2012). Reevaluation of host specificity of the closely related species *Pseudoperonospora humuli* and *P. cubensis*. Plant Disease 96(1), 55-61.

Sackston, W.E., Gulya, T.J., Miller, J.F. (1990): A proposed international system of designating races of *Plasmopara halstedii*. Plant Disease 74(9), 721-723.

Sackston, W.E., Vimard, B. (1988): Leaf disc immersion (LDI) inoculation of sunflower with *Plasmopara halstedii* for in vitro determination of host-pathogen relationships. Plant Disease 72, 227-229.

Sackston, W.E. (1981): The sunflower crop and disease: progress, problems, and prospects. Plant Disease 65(8), 643-648.

Samoucha, Y., Cohen, Y. (1984): Synergy between metalaxyl and mancozeb in controlling downy mildew in cucumbers. Phytopathology 74(12), 1434 – 1437.

Schornack, S., Huitema, E., Cano, L.M., Bozkurt, T.O., Oliva, R., van Damme, M., Schwizer, S., Raffaele, S., Chaparro-Garcia, A., Farrer, R., Segretin, M.A., Bos, J., Haas, B.J., Zody, M.C., Nusbaum, C., Win, J., Thines, M., Kamoun, S. (2009): Ten things to know about oomycete effectors. Molecular Plant Pathology 10(6), 795-803.

Sedlář, J., Sedlářová, M., Flusser, J. (2009): Image processing methods for determination of downy mildews from light microscopy images. In: Proceedings of Signal Processing Symposium. Warsaw, Poland. [http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.486.718&rep=rep1&type=pdf]. Accessed 20 March 2017.

Sedlářová, M., Pospíchalová, R., Drábková Trojanová, Z., Bartůšek, T., Slobodianová, L., Lebeda, A. (2016): First report of *Plasmopara halstedii* new races 705 and 715 on sunflower from Czech Republic – short communication. Plant Protection Science 52(3), 182-187.

Sedlářová, M., Trojanová, Z., Lebeda, A. (2013): Distribution and harmfulness of *Plasmopara halstedii* on sunflower in the Czech Republic. Plant Protection Science 49(1), 1-10.

Sedlářová, M., Stojaspal, K., Lebeda, A. (2010): Rozšíření a patogenita *Plasmopara halstedii*, původce plísně slunečnice, v České republice. Rostlinolékař 1, 17-20.

Seiler, G. (1992): Wild sunflower as a potential source of resistance to downy mildew. In: Gulya T.J., Vear, F. (Eds.): ISA Symposium III sunflower downy mildew. Fargo, ND, USA: International sunflower association, pp. 113-121. [http://isasunflower.org/fileadmin/documents/Symposia/3rd_Symposium_on_Sunflower_Mildew__USA__1998/T03-113_-Wild_Sunflower_as_a_Potential_Source_of_resistance_to_Downy_Mildew.pdf]. Accessed 20 March 2017.

Sharma, R., Xia, X., Cano, L. M., Evangelisti, E., Kemen, E., Judelson, H., Oome, S., Sambles, C., van der Hoogen, D.J., Kitner, M., Klein, J., Meijer, H.J.G., Spring, O., Win., J., Zipper, R., Bode, H.B., Govers., F., Kamoun. S., Schornack, S., Studholme, D.J., van der Ackerveken, G., Thines, M. (2015): Genome analyses of the sunflower pathogen *Plasmopara halstedii* provide insights into effector evolution in downy mildews and *Phytophthora*. BMC Genomics 16(1), 741.

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.

Skidmore, D.I., Ingram, D.S. (1985): Conidial morphology and the specialization of *Bremia lactucae* Regel (Peronosporaceae) on hosts in the family Compositae. Botanical Journal of the Linnean Society 91(4), 503-522.

Slobodianová, L. (2016): Variabilita populací *Plasmopara halstedii* v ČR – molekulární metody. Diploma thesis, Deposited at Department of Botany, Faculty of Science, Palacký University in Olomouc, Czech Republic.

Spring, O. (2009): Transition of secondary to systemic infection of sunflower with *Plasmopara halstedii* – an underestimated factor in the epidemiology of the pathogen. Fungal Ecology 2(2), 75-80.

Spring, O., Zipper, R. (2006): Evidence for asexual genetic recombination in sunflower downy mildew, *Plasmopara halstedii*. Mycological Research, 110(6), 657-663.

Spring, O., Bachofer, M., Thines, M., Riethmüller, A., Göker, M., Oberwinkler, F. (2006): Intraspecific relationship of *Plasmopara halstedii* isolates differing in pathogenicity and geographic origin based on ITS sequence data. European Journal of Plant Pathology 114(3), 309–15.

Spring, O., Voglmayr, H., Riethmüller, A., Oberwinkler, F. (2003): Characterization of *Plasmopara* isolate from *Helianthus* x *laetiflorus* based on cross infection, morphological, fatty acids and molecular phylogenetic data. Mycological Progress 2(3), 163-170.

Spring, O., Haas, K. (2002): The fatty acid composition of *Plasmopara halstedii* and its taxonomic significance. European Journal of Plant Pathology 108(3), 263-267.

Spring, O. (2001): Nonsystemic infection of sunflower with *Plasmopara halstedii* and their putative role in the distribution of the pathogen. Journal of Plant Diseases and Protection 108(4), 329-336.

Spring, O. (2000): Homothallic sexual reproduction in *Plasmopara halstedii*, the downy mildew of sunflower. Helia 23(32), 19-26.

Spring, O., Rozynek, B., Zipper, R. (1998): Single spore infections with sunflower downy mildew. Journal of Phytopathology 146(11-12), 577-579.

Spring, O., Rozynek, B., Zipper, R. (1997): Leaf disc inoculation – a useful tool for selecting infections of sunflower downy mildew at low inoculum concentration, but inappropriate to pathotype characterization. Journal of Phytopathology 145(4), 189-191.

Spring, O., Miltner, F., Gulya, T.J. (1994): New races of sunflower downy mildew (*Plasmopara halstedii*) in Germany. Journal of Phytopathology 142(3), 241-244.

Spring, O., Benz, A., Faust, V. (1991): Impact of downy mildew (*Plasmopara halstedii*) infection on the development and metabolism of sunflower/Auswirkungen der Infektion mit Falschem Mehltau (*Plasmopara halstedii*) auf die Entwicklung und den Stoffwechsel von Sonnenblumen. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 98(6), 597-604.

Spurný M. (2005): Hybridy slunečnice Rustica úspěšné v boji proti plazmopaře. In: Řepka, mák, slunečnice a hořčice. Česká zemědělská společnost na ČZU v Praze a Katedra rostlinné výroby, Prague, Czech Republic, pp. 164–166.

Sudisha, J., Niranjana, S.R., Sukanya, S.L., Girijamba, R., Devi, N.L., Shetty, H.S. (2010): Relative efficacy of strobilurin formulations in the control of downy mildew of sunflower. Journal of Pest Science 83(4), 461-470.

Thines, M. (2014): Phylogeny and evolution of plant pathogenic oomycetes - a global overview. European Journal of Plant Pathology 138(3), 431-447.

Thines, M., Runge, F., Telle, S., Voglmayr, H. (2010): Phylogenetic investigations in the downy mildew genus *Bremia* reveal several distinct lineages and a species with a presumably exceptional wide host range. European Journal of Plant Pathology 128(1), 81-89.Thines, M. (2007): Use of scanning electron microscopy in downy mildew systematics. In: Lebeda, A., Spencer-Phillips, P.T.N. (Eds.): Advances in Downy Mildew Research. Kostelec na Hané: JOLA and Palacký University in Olomouc, Czech Republic, pp. 17-23.

Thines, M., Göker, M., Oberwinkler, F., Spring, O. (2007): A revision of *Plasmopara penniseti*, with implications for the host range of the downy mildews with pyriform haustoria (DMPH). Mycological Research 111(12), 1377-1385.

Tosi, L., Beccari, G. (2007): A new race, 704, of *Plasmopara helianthi* pathogen of sunflower downy mildew in Italy. Plant Disease 91(4), 493-493.

Tourvieille de Labrouhe, D., Walser, P., Jolivot, D., Roche, S., Serre, F., Leguillon, M., Vear, F. (2012): Proposal for improvement of sunflower downy mildew race nomenclature. In: Proceedings of the 18th International Sunflower Conference. Mar del Plata, Argentina, pp. 322-327.

Tourvieille De Labrouhe, D., Serre, F., Walser, P., Roche, S., Vear, F. (2008a): Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*). Euphytica 164(2), 433-444.

Tourvieille de Labrouhe, D., Walser, P., Serre, F., Roche, S., Vear, F. (2008b): Relations between spring rainfall and infection on sunflower by *Plasmopara halstedii* (downy mildew). In: Velasco, L. (ed.) Proceedings of the 17th International Sunflower Conference Vol. 1. Cordoba, Spain, pp. 97-102.

Tourveille de Labrouhe, D., Lafon, S., Walser, P., Raulic, I. (2000a): A new strain of *Plasmopara halstedii*, a form of mildew that attacks sunflowers. Ocl-Oleagineux Corps Gras Lipides 7(5), 404-405.

Tourvieille de Labrouhe, D., Gulya, T.J., Masirevic, S., Penaud, A., Rashid, K., Virányi, F. (2000b): New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). In: Proceedings of the 15th International Sunflower Conference. Toulouse, France: International Sunflower Association, pp. 61-66.

Trojanová, Z., Sedlářová, M., Gulya, T.J., Lebeda, A. (2017): Methodology of virulence screening and race characterization of *Plasmopara halstedii*, and resistance evaluation in sunflower – a review. Plant Pathology 66, 171-185.

Trojanová, Z., Sedlářová, M., Bartůšek, T., Stojaspal, K., Lebeda, A. (2013): Studium populace *Plasmopara halstedii* na území ČR v letech 2007-2013, rozšíření a variabilita. Mykologické listy 125, 36.

Trojanová, Z. (2010): Patofyziologie slunečnice: interakce s *Plasmopara halstedii*. Diplomma thesis, Deposited at Department of Botany, Faculty of Science, Palacký University in Olomouc, Czech Republic.

Turner, J.A. (2015): The Pesticide Manual: A World Compendium, 17th edition. British Crop Protection Council (BCPC), Alton, Hampshire, UK, 1357 p.

Vear, F. (2016): Changes in sunflower breeding over the last fifty years. OCL, 23(2), D202. [http://www.ocl-journal.org/articles/ocl/full_html/2016/02/ocl160006-s/ocl160006-s.html]. Accessed 20 March 2017.

Vear, F., Serre, F., Roche, S., Walser, P., Tourvieille de Labrouhe, D. (2007): Recent research on downy mildew resistance useful for breeding industrial-use sunflowers. Helia 30(46), 45-54.

Vear, F. (2004): Breeding for durable resistence to the main diseases of sunflower. In: Proceedings of the 16th International sunflower conference. Fago, ND, USA, pp. 15-28.

Veverka, K., Křížková-Kudlíková, I. (2006): Problematika testování odolnosti hybridů slunečnice vůči houbovým chorobám na provokačním pozemku. In: XVIIth Czech and Slovak Conference on Plant Protection (Book of Abstracts). Czech Agricultural University, Prague, pp. 287-288.

Vincourt, P., As-sadi, F., Bordat, A., Langlade, N.B., Gouzy, J., Pouilly, N., Lippi, Y., Serre, F., Godiard, L., Tourvieille de Labrouhe, D., Vear, F. (2012): Consensus mapping of major resistance genes and independent QTL for quantitative resistance to sunflower downy mildew. Theoretical and Applied Genetics 125(5), 909-920.

Virányi, 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(63), 149-162.

Virányi, F., Spring, O. (2011): Advances in sunflower downy mildew research. European Journal of Plant Pathology 129(2), 207-220.

Virányi, F. (2008): Research progress in sunflower diseases and their management. In: Proceedings of the 17th International Sunflower Conference, Córdoba, Spain, pp. 1-12. [http://isasunflower.org/fileadmin/documents/aaProceedings/17thISC_CordobaVol1/1Viranyi.pdf]. Accessed 12 March 2017

Virányi, F. (2002): The sunflower-*Plasmopara halstedii* pathosystem: natural and artificially induced coevolution. In: Spencer-Philips, P.T.N, Gisi, U., Lebeda, A. (Eds.): Advances in Downy Mildew Research. Volume 1. Kluwer Academic Publisher, London, UK, pp. 167-172.

Virányi, F., Gulya, T.J. (1995): Inter-isolate variation for virulence in *Plasmopara halstedii* (sunflower downy mildew) from Hungary. Plant Pathology 44(4), 619-624.

Virányi, F. (1988). Plasmopara halstedii. European handbook of plant diseases, pp. 228-230.

Voglmayr, H., Constantinescu, O. (2008): Revision and reclassification of three *Plasmopara* species based on morphological and molecular phylogenetic data. Mycological Research 112(5), 487-501.

Voglmayr, H., Fatehi, J., Constantinescu, O. (2006): Revision of *Plasmopara* (Chromista, Peronosporales) parasitic on Geraniaceae. Mycological Research 110(6), 633-645.

Walcz, I., Bogár, K., Virányi, F. (2000): Study on *Ambrosia* isolate of *Plasmopara halstedii*. Helia 23(33), 19-24.

Waterhouse, G.M. (1973): Peronosporales. In: Ainsworth, G.C., Sparrow, F.K., Sussman, A.S. (Eds.): The Fungi. An advanced treatise. Academic Press, New York, pp. 165–183.

Wilson, G.W. (1908): Studies in North American Peronosporales – IV. Host Index. Bulletin of the Torrey Botanical Club 35(11), 543-554.

Yokoi, T., Yamashita, S., Hibi, T. (2003): The nucleotide sequence and genome organization of *Sclerophthora macrospora* Virus A. Virology 311(2), 394–399.

Yokoi, T., Takemoto, Y., Suzuki, M., Yamashita, S., Hibi, T. (1999): The nucleotide sequence and genome organization of *Sclerophthora macrospora* Virus B. Virology 264(2), 344–349.

Young, P.A., Morris, H. E. (1927): *Plasmopara* downy mildew of cultivated sunflowers. American Journal of Botany, 14(9), 551-552.

Zahka, G.A., Viranyi, F. (1991): Axenic culture of the downy mildew fungus *Plasmopara halstedii* in *Agrobacterium rhizogenes*-induced roots of sunflower (*Helianthus annuus*). Canadian Journal of Botany 69(12), 2709-2715.

Zimmer, D.E. (1974): Physiological specialization between races of *Plasmopara halstedii* in America and Europe. Phytopathology 64(11), 1465-1467.