Eur J Plant Pathol (2011) 129:207–220
DOI 10.1007/s10658-010-9683-0

Advances in sunflower downy mildew research

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Accepted: 6 September 2010 / Published online: 16 September 2010

Abstract This review summarises the progress in research on sunflower downy mildew as reported in publications of the past 10 to 15 years, the period since the last comprehensive review on *Plasmopara halstedii*. Particular attention is paid to subjects that showed much progress and may be of particular interest to sunflower pathologists, mycologists or molecular biologists. Accordingly, single sections are devoted to the problems of taxonomic and phylogenetic aspects, host specificity, the host—pathogen interaction including resistance phenomena, as well as epidemiology and disease management. Reflecting the progress achieved in some fields and illuminating the deficits in others should stimulate the reader's interest in this very significant pathosystem.

Keywords *Plasmopara halstedii* · Taxonomy · Host range · Resistance · Phenotypic and genotypic diversity · Epidemiology

Introduction

Downy mildew of sunflower, incited by *Plasmopara halstedii* (Farl.) Berlese et de Toni, is considered one of the most severe pathogenic threats of this crop worldwide. Since the pathogen shows high phenotypic diversity, particularly in virulence and fungicide sensitivity, effective disease control depends on profound knowledge of the biology of the pathogen, its physiological capacities and requirements as well as the molecular mechanisms involved in the interaction with the host and environment. In a previous review, Gulya and co-workers (1997) summarized in detail the state of knowledge in the biology, host—pathogen relationships and disease control of sunflower downy mildew.

However, since then significant progress has been made in research and a number of publications have presented new findings and ideas. There is a need, therefore, to outline the advances in research over the last decade with particular emphasis on the taxonomic position, host specialisation and genetics of this oomycete, coupled with resistance phenomena, epidemiology and disease management. Accordingly, in this review we have chosen to elaborate in detail the highlights of this pathosystem, with the aim of providing up-to-date information to those interested in this devastating disease.

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Historical and phylogenetic aspects of sunflower downy mildew taxonomy

The taxonomic situation of *Plasmopara halstedii* has previously been illuminated in various reviews (e.g., Zimmer and Hoes 1978; Sackston 1981; Viranyi 1992; Gulya et al. 1997). However, the scientific information contributed from East European scientists to this field

particularly within the first two decades after World War II and summarized by Novotelnova (1966) appears to be inadequately considered. Hence, there is an ongoing discussion on the correct classification and the species delineation since the first description of the pathogen.

Farlow (1883) described the downy mildew pathogen *Peronospora halstedii* on the basis of pathogen samples found on *Eupatorium purpureum, Ambrosia artemisiifolia, Bidens frondosa, Rudbeckia laciniata, Silphium terebinthaceum* and the perennial sunflower species *Helianthus strumosus, H. tuberosus and H. doronicoides* (*H. mollis X giganteus*). It appears noteworthy that he first referred to a sample Halsted found on *Eupatorium purpureum* near the Bussey Institution in 1876 and it can be deduced that he had this specimen (Farlow Herbarium #79717) in mind when he stated that a sample found on *H. tuberosus* by Ellis showed "…the most marked deviation from the type…". When Schröter (1886) had separated *Plasmopara* from *Peronospora* due to the germination by means of zoospores instead of forming germ tubes, Berlese and de Toni (1888) transferred the taxon to the new genus under the name *Plasmopara halstedii* (Farlow) Berlese & de Toni. In their description, the host range comprised seven genera, including four perennial species of *Helianthus*, but again no annuals. Based on subtle morphological differences, Wilson (1907) described the genus *Rhysotheca* in which *Plasmopara halstedii* had been embedded, but the new genus never gained broad acceptance. Over the subsequent years, numerous collections of downy mildews on species of Asteraceae were made in North America and classified as *Plasmopara halstedii*, due to morphological similarities of sporangiophores and sporangia, although high variability in size and shape was recognized early (Stevens 1913). The first specimens documenting the occurrence of downy mildew on *Helianthus annuus* were dated from the 1890s and in the 1920s it became a serious threat to sunflower production in many states of the U.S. (Henry and Gilbert 1924; Young and Morris 1927).

The pathogen was considered "…to occur on almost any composite …" (Nishimura 1922). This was in contrast to Gäumann's narrow host-based species concept (1923) and to the situation found in most species of the Peronosporaceae according to the current classification. Frequently *Plasmopara halstedii* was regarded as a species complex (Leppik 1966). Clear evidence for the conspecificity of the pathogen originally described by Farlow with isolates found on cultivated sunflower *H. annuus* is still lacking. In Eastern Europe, where sunflower downy mildew became a serious threat in the middle of the 20th century, intensive cross infection studies with pathogen inoculum from oilseed sunflower were performed to elucidate the host range of sunflower downy mildew (reviewed by Novotelnova 1966).

Field experiments never showed disease symptoms on host plants other than *H. annuus* and a few other annual species of the genus *Helianthus*. This encouraged Novotelnova to reclassify the species complex (Novotelnova 1962, 1964, 1966). She retained Farlow's name *Plasmopara halstedii* for pathogens found on Eupatorieae, described several new species on hosts from other tribes and separated the downy mildew of sunflower as *Plasmopara helianthi* Novot. Within the latter she further differentiated the specialized forms *helianthi*, *perennis* and *patens* based on natural and artificial infection studies with annual or perennial *Helianthus* species and on slight morphometric differences in sporangia, respectively. However, her species concept never gained broad acceptance and sunflower downy mildew is still mostly known as *Plasmopara halstedii*.

Fifty years later it appears possible and necessary to test the conspecifity for Farlow's *P. halstedii* and Novotelnova's *P. helianthi*. Molecular genetic tools have fundamentally influenced our understanding of the oomycete phylogeny and changed the classification at all taxonomic levels (reviewed by Voglmayr 2008, Spring and Thines 2004). Moreover, on the basis of phylogenetic studies, new phenotypic characters were searched for and evaluated for reliability (Spring and Thines 2004; Thines 2006).

Plasmopara in the sense of Schröter (1886) was recognised as a polyphyletic entity which, in past years, has been separated into a core genus (harbouring *P. halstedii*) and several new genera (for references see Voglmayr and Constantinescu 2008). A broad survey on molecular genetic data from downy mildews on Asteraceae is still lacking due to technical problems in getting reliable DNA sequence information from old desiccated material compiled in herbarium collections. However, new methodologies to overcome such restrictions are in progress (Telle and Thines 2008). The first data obtained from herbarium specimens of downy mildew infected Asteraceae (Thines, personal

communication) clearly indicate that Farlow's suggestion to separate *P. halstedii* on Tubuliflorae from *P. ganglioniformis* (= *Bremia lactucae*) on Liguliflorae is insufficient as a species concept for downy mildew pathogens of this plant family. Recent phylogenetic results gradually resolve the large downy mildew complex living on Asteraceae and confirm separate entities on the generic (e.g. *Bremia*, *Novotelnova, Paraperonospora*, *Plasmopara, Protobremia*) and species level (Voglmayr and Constantinescu 2008). Narrow species concepts such as those found in *Novotelnova scorzonerae*, *Paraperonospora leptosperma*, *Plasmopara angustiterminalis* or *Plasmopara* on *Flaveria bidentis* are supported by the new characters analysed (Spring et al. 2003; Thines 2006; Komjati et al. 2007; Voglmayr and Constantinescu 2008). In this context, recent studies on downy mildew of *Xanthium strumarium* (Komjáti et al. 2007) in Hungary and on a naturalized population of the perennial sunflower hybrid *Helianthus* x *laetiflorus* (Spring et al. 2003) revealed that the former is correctly classified as *P. angustiterminalis* Novotelnova while the latter was indistinct from downy mildew samples collected from annual oilseed sunflower. The fact that both isolates readily infected *H. annuus* seedlings when sporangia were used as inoculum is questioning Novotelnova's (1966) concept of differentiating specific forms of *P. helianthi* according to infection patterns on annual or perennial sunflower species.

Host range and distribution of sunflower downy mildew

The unresolved taxonomic situation is directly linked with questions on the true host range of sunflower downy mildew, its geographic origin and current distribution. While former assumptions of a broad host range of *P. halstedii* were based on morphological similarities of downy mildew pathogens found on Asteraceae (Farlow 1883; Nishimura 1922; Leppik 1966), infection studies performed by East European pathologists with inoculum from sunflower downy mildew revealed a very narrow host range for this pathogen (reviewed by Novotelnova 1966). In tests with more than 70 potential hosts under field and artificial conditions, only the annual *H. lenticularis* (a western variety of wild *H. annuus*) and *H. debilis* as well as the perennial sunflower species *H. maximiliani*, *H. grosseserratus* and *H. tomentosus* (syn. *H. tuberosus*) and the hybrid *Heliathus* x *multiflorus* were susceptible (Christova and Mitov 1960; Pustovoit 1963, 1964; Novotelnova 1966). This list was expanded by Leppik (1966) and Virányi (1984) who succeeded to infect *H. divaricatus* and *H. argophyllus*, respectively, with zoosporangia gained from oilseed sunflower. To date, infection of species from other genera of Asteraceae has not been achieved with such inoculum.

It must be emphasized that previous infection tests were predominantly carried out using only inoculum from cultivated *H. annuus*. Experiments testing the susceptibility of cultivated *H. annuus* to inocula from other Asteraceae have been reported to a much lesser extent. This is due to the problem that such experiments continuously require fresh inoculum and that the culture of pathogen strains isolated from wild species on their original hosts is difficult to establish. Infection of *H. annuus* succeeded with zoosporangia from a pathogen on *Helianthus* x *laetiflorus*, found on a naturalized colony of the perennial host in Germany (Spring et al. 2003). The pathogen survived in the rhizome of its host, leading to yearly systemic infection of shoots and leaves. Based on morphological, chemical and molecular genetic characters, the pathogen was indistinct from strains of downy mildew found on cultivated annual sunflower. Interestingly, its infection behaviour on sunflower differential lines did not allow the assignment of a certain pathotype, because several of the commonly used sunflower differential lines (Tourvieille et al. 2000) died from damping-off before sporulation occurred. This may suggest that the pathogen was introduced with its perennial host rather than being spread from local sunflower fields.

Possible host jumps of downy mildew pathogens of other Asteracean genera to *H. annuus* were reported from *Ambrosia artemisiifolia* (Walcz et al. 2000) and from *Xanthium strumarium* (Virányi 1984; Komjáti et al. 2007) in Hungary and from *Iva xanthifolia* in the U.S. (Gulya 2002a). In all three cases, successful cross-infection under laboratory conditions was achieved using zoosporangia of the wild hosts for whole seedling inoculation (Cohen and Sackston 1973) of sunflower. However, natural infection of cultivated sunflower with sporangia of downy mildew from wild Asteracean host species has not yet been proven under field conditions. In the case

of the reported *X. strumarium* incidence, the pathogen could be maintained on certain sunflower genotypes (e.g. the differential line QHP 1), but killed others by early damping-off. Molecular genetic and other characters undoubtedly identified this pathogen as *P. angustiterminalis* Novot. (Komjáti et al. 2007; 2008), a species phylogenetically close to *P. halstedii* (Voglmayr et al. 2004). Such molecular genetic investigations are lacking for the pathogens found on *Ambrosia* and *Iva*, but could still be performed if herbarium specimens are available.

The fact that two species of *Plasmopara* potentially infect the same host is of particular interest, because it could provide the possibility for hybridization as previously shown for oomycetes (Adhikari et al. 2003; Schardl and Craven 2003; Spring and Zipper 2006; Érsek and Nagy 2008).

As reviewed earlier by Sackston (1981), the origin of the sunflower downy mildew pathogen is seen in North America, where its host *H. annuus* is one of the species of *Helianthus* with the widest geographic distribution (Heiser 1969; Rogers et al. 1982). On wild sunflower, downy mildew infection is remarkably rare (the US National Fungus Collection, BPI, stores specimens of 11 *Helianthus* species infected with downy mildew, while the genus is comprised of 49 species) and epidemics on wild populations have never been reported. This makes wild *H. annuus* a potential source for disease resistance and raises the question of how and from where did sunflower downy mildew start its world-wide spread.

Although nutritional use of sunflower is known from Indian tribes in North America since ca. 5,000 years (for review see Putt 1997) and sunflower varieties selected by the Havasupai and Hopi Indians are assumed to be the potential origin of the modern cultivated crop (Heiser 1976; Gulya 1992), intensive sunflower agriculture did not originate in the U.S., but was initiated in the late 19th century by Russian emigrants. It is noteworthy that downy mildew was not known from Europe until the 1940s when *P. halstedii* occurred in former Yugoslavia and later in other East European countries (for review see Sackston 1992), while first reports of sunflower downy mildew in North American sunflower fields are from the early 20th century (Young and Morris 1927; Bisby et al. 1938). This makes very unlikely the host jump from a downy mildew pathogen of wild European Asteraceae (e.g. *X. strumarium*) to cultivated sunflower and supports Sackston's (1992) theory of the North American origin of sunflower downy mildew. Most likely the export of unnoticed contaminated seeds (Meliala et al. 2000; Ioos et al. 2007) gave rise to the worldwide distribution of sunflower downy mildew.

Consecutive development of numerous pathotypes appears to be the natural result of sunflower breeding and subsequent selection of compatible pathogen genotypes rather than the result of independent acquisition from natural populations endemic to different geographic regions of the world. On the other hand, introduced populations of the pathogen may have subsequently evolved in different areas of the world to form the numerous pathotypes. Detailed molecular genetic studies on this topic are still lacking, but a recent investigation on French isolates from 14 pathotypes indicates that multiple introductions of different pathotypes in combination with selection pressure exerted by host resistance genes may have accelerated the evolution of pathotype diversity in France (Delmotte et al. 2008).

In this context it is noteworthy, that partial sequencing of a RNA mycovirus from sunflower downy mildew revealed identical nucleotide sequences in all *Plasmopara halstedii* strains of eight pathotypes originating from eight countries in three continents (Heller-Dohmen et al. 2008). This suggests contamination of the sunflower downy mildew pathogen with the virus before its worldwide distribution and pathotype segregation.

Genetic diversity—origin and molecular genetic aspects

Numerous attempts have been made in the past to differentiate sunflower downy mildew pathotypes by means of morphological and molecular genetic characters, but infection of sunflower differential lines is still the only practical test for pathotype classification. Nevertheless, molecular genetic investigations revealed a high level of genetic variability which is the basis for pathotyping.

The early applied techniques such as Restriction Fragment Length Polymorphism (RFLP) (Borovkov and McClean 1993), RAPD (Borovkova et al. 1992; Roeckel-Drevet et al. 1997), and Inter Simple Sequence Repeats (ISSR) (Intelmann and Spring

2002) analysis indicated genetic diversity between the samples investigated, but were insufficient to differentiate populations on the intraspecific level. Sequencing of the large subunit of ribosomal DNA was helpful to resolve the phylogeny of the Peronosporaceae including the genus *Plasmopara* (Riethmüller et al. 2002; Voglmayr et al. 2004) but was inappropriate to resolve intraspecific groups of *P. halstedii*. For the latter, the less conserved internal transcribed genomic region (ITS) appeared to be a more promising tool. In particular, the ITS2 region of *P. halstedii* attracted interest due to its enormous size in comparison to other Peronosporaceae (Thines et al. 2005a). This length polymorphism is caused by inserted repetitive elements, representing a synapomorphy of downy mildews with pyriform haustoria (Thines et al. 2005b). It is not only useful for interspecific phylogenetic studies, but is promising for investigations at the population level (Thines 2007a, b). Based on differences in partial sequence analysis of the nuclear ITS region, Spring et al. (2006) finally succeeded to differentiate between certain pathotypes of sunflower downy mildew originating from different geographic regions.

More recently, molecular genetic studies based on single nucleotide polymorphisms (SNPs) and size variation (insertions/deletions) in expressed sequence tags (ESTs) were performed using sunflower downy mildew isolates from France and Russia (Giresse et al. 2007). The authors found high genetic variability and the technique seems to be appropriate for high-throughput genotyping. Their current results showed a low level of heterozygosity, hence indicating that *P. halstedii* is a selfing species. This is concordant with the homothallic gametangiogamy found in single zoospore infection of sunflower with the pathogen (Spring 2000). On the other hand, homothally raises questions on the processes generating the observed genetic and phenotypic variability in sunflower downy mildew (Delmotte et al. 2008). Besides sexual recombination by occasional outcrossing, numerous mechanisms such as mitotic recombination, gene conversion, transposable elements or dispensable chromosomes were found to account for the genetic variability in other oomycetes (Chamnanpunt et al. 2001; Kamoun 2003). In addition, zoospore fusion was found to create asexual hybrids in *Phytophthora* species (Érsek et al. 1995; Érsek and Nagy 2008). Dual infections of sunflower with downy mildew strains differing in pathogenicity and fungicide tolerance have recently resulted in mitotically derived recombinant offspring, hence giving evidence for asexual genetic recombination in *P. halstedii* (Spring and Zipper 2006). More recent experiments showed that such unusual genetic recombination may even involve different species of *Plasmopara*, as long as they are able to infect the same host tissue (Hammer 2009). Although the mode of gene transfer has not yet been clarified, anastomosis appears to be a possible way to transfer nuclei, mitochondria or just DNA fragments between hyphae of two individuals (Hammer and Spring 2006). For unequivocal proof of gene transfer via anastomosis, visual staining for microscopic identification of the participating mycelia is required, but so far, attempts to transform sunflower downy mildew have resulted in transient expression only (Hammer et al. 2007). Similar experiences have been reported for experiments to transform another biotrophic pathogen of the genus, *P. viticola* (Dubresson et al. 2008). In this case, *gfp* expression in grape downy mildew lasted over four generations.

Pathogen-host interaction, resistance phenomena

The interaction between sunflower and *Plasmopara halstedii* can be described with a typical gene-for-gene relationship where the different pathotypes each having specific virulence character will meet sunflower genotypes with or without effective resistance gene(s) against them. Although genetic resistance in sunflower to *P. halstedii* has long been described, and a series of Pl genes identified and incorporated into downy mildew resistant sunflower cultivars, there remained many questions to be answered in order to effectively control this devastating sunflower disease over the long term. In fact, researchers in different countries are working hard to understand better the mechanism, as well as the genetic and molecular basis of resistance, searching for new genes or gene clusters conferring resistance, selecting for such genes using molecular markers in breeding programmes and looking for alternative ways to confer resistance in sunflower.

Expression of resistance

P. halstedii infection of sunflower usually takes place in below ground plant parts by directly penetrating root and/or hypocotyl tissues resulting in systemic

downy mildew infection. It had already been shown that chemical stimuli secreted by the roots attract zoospores that move towards the root surface, thus enhancing successful penetration (Delanoe 1972). Unfortunately, there are no data available so far as to whether or not any difference exists in the reactions of either compatible or incompatible interactions regarding this phenomenon nor have such signals been identified.

Postpenetration events in sunflower may vary depending on the pathogen–host interaction, i.e. the type of resistance (Mouzeyar et al. 1993; Virányi and Gulya 1996). Accordingly, the host may strictly stop pathogen development at or near the penetration site, or alternatively, it may allow colonization of root, hypocotyl or even cotyledon tissues. However, in most cases, resistance is associated with restricted or intensive host cell death. In other words, sunflower reacts to infection with a hypersensitive reaction (HR) a well-known resistant host response to pathogen attack (Virányi 1980; Mouzeyar et al. 1993). However, the genetic background of this phenomenon was not known. Recently, Herbette et al. (2003) suggested the involvement of GPX (glutathione peroxidase) enzymes in the hypersensitive response in sunflower and Radwan et al. (2005) using Real Time Polymerase Chain Reaction (RT-PCR) analysis showed that resistance was associated with the activation of a hsr203J-like gene, a molecular marker of HR in tobacco. Activation of this gene was specifically observed during the incompatible interaction and coincided with cell collapse in sunflower hypocotyls. Interestingly, this reaction is tissue specific and did not occur in leaves. It may, therefore, explain, at least in part, the observed types of latent and/or local infections of sunflower. The former allows the pathogen to survive and even sexually reproduce in roots (Heller et al. 1997) and/or hypocotyls (Virányi 1980) of virtually resistant host genotypes, while the latter prohibited pathotype differentiation on leaf discs (Rozynek and Spring 2001) and may give rise to floral infection which contributes to pathogen dissemination through apparently healthy seeds (Meliala et al. 2000; Spring 2001).

In a recent study, Chaki et al. (2009) investigated the role of reactive oxygen and nitrogen species (ROS, RNS) in sunflower susceptible or resistant to *P. halstedii* by using confocal laser scanning microscopy (CLSM) and biochemical analyses. They analyzed the superoxide radical production, hydrogen peroxide content, L-arginine-dependent nitric oxide synthase and S-nitrosoglutathione reductase activities, and their location. Their results showed that the pathogen induced nitrosative stress in the susceptible but not in the resistant sunflower.

Resistance genes, gene clusters

Considerable efforts had been undertaken since the 1990s to locate resistance genes in the sunflower genome and to use the knowledge in plant breeding. In an earlier study, Vear et al. (1998) using RFLP markers pointed out that Pl4 appears not linked or allelic with Pl2 and that Pl5 is not located in the cluster including Pl1, Pl2 and Pl6. Similarly, Brahm et al. (1998) could construct linkage maps that represented the genomic region carrying the respective resistance loci. By homology cloning, Gentzbittel et al. (1998) obtained probes by which they demonstrated that at least three NBS-like loci were located on linkage group 1, i.e. in the region where downy mildew resistance loci had been described. More recently, Radwan et al. (2004) in France and Dußle et al. (2004) in Germany achieved considerable results with PCR markers for the Pl5/Pl8 locus from complete CC-NBS-LLR sequences and with the localization of the Pl_{ARG} gene using SSR markers, respectively. Furthermore, Pankovic et al. (2007) developed two co-dominant cleaved amplified polymorphic sequence (CAPS) markers that completely co-segregated with the Pl6 gene conferring resistance to pathotype 730. They concluded that CAPS markers will facilitate efficient marker-assisted selection for sunflower resistance to downy mildew pathotype 730.

Sources and inheritance of resistance

The frequency of Pl genes in wild sunflower species is high, especially in the annual species, some of these having resistance for all known pathotypes. Whereas it may occur that a single species has several genes to control a single pathotype (Seiler 1998), Terzic et al. (2007) included in their study 29 populations of five wild annual sunflowers that were crossed with cultivated ones to find out new sources of resistance to downy mildew. Their results suggested that the annual wild sunflower species, particularly *H. annuus* and *H. argophyllus,* could be used to obtain such

resistance. Inheritance studies carried out in Spain (Molinero-Ruiz et al. 2003) and in Serbia (Pankovic et al. 2005) documented that resistance to pathotypes 310 and 703, or to pathotype 730 were controlled by single dominant genes.

Quantitative or non-race-specific resistance

Genetic resistance of sunflower to downy mildew, conferred by major gene(s) is effective but will be overcome by the appearance of new pathogenic forms of *P. halstedii* possessing an altered virulence profile. Within a new breeding programme recently started in France, Tourvieille de Labrouhe et al. (2005) showed that pyramiding, alternation or mixtures of a few major genes appeared to increase the duration of effective control of downy mildew. As an alternative, resistance of sunflower which does not depend on the host/pathogen interaction has been studied by Vear et al. (2006) in large scale trials for 3 years. A significant level of partial resistance was shown in cultivated sunflower lines that do not have Pl genes effective against the pathotypes present. This resistance appeared independent of pathogen virulence phenotype and its heredity was under additive control. This type of quantitative resistance was also tested by Serre et al. (2008) under growth chamber conditions and the importance of plant age and environment interaction during host resistance response was pointed out. Partial resistance to *P. halstedii* in high oleic sunflower hybrids has been reported as well by Baldini et al. (2006) in Italy. Quite recently, in summarizing their 4-year-study, Tourvieille de Laboruhe et al. (2009) suggested that it should be possible to select for non-race-specific downy mildew resistance and to include it in modern varieties. However, since this type of resistance is partial, it may be necessary to combine it with major gene resistance.

Induced resistance

Apart from genetic and durable (quantitative/non-race-specific/multiple) resistance, chemically induced resistance might be considered as an additional and useful element in the integrated management against sunflower downy mildew. The phenomenon has already been known for years (reviewed by Sticher et al. 1997) and the plant activator benzothiadiazole (BION 50 WP as commercial product) has been used successfully against powdery mildew of wheat. In addition, researchers from Hungary and Italy also reported the positive effect of this compound in depressing downy mildew symptoms under field and greenhouse conditions, respectively (Tosi et al. 1999; Bán et al. 2004); and a remarkable increase in induced resistance against broomrape attack by *Orobanche cumana* was evident due to benzothiadiazole treatment of sunflower in Germany (Sauerborn et al. 2002). Furthermore, microscopical observations on treated and inoculated sunflower tissues revealed well-defined and characteristic alterations as host responses to infection and such changes resembled those occurring with Pl-gene mediated resistant plants following infection (Bán et al. 2004). Investigations in different laboratories have been focusing on the biochemical and molecular genetic background of this induced resistance mechanism. For example, Serrano et al. (2007) in Spain reported an increased peroxidase and chitinase activity in BTH-treated and inoculated susceptible and resistant sunflower seedlings. In Hungary, investigations have been made on the expression of genes allegedly associated with resistance response by measuring the accumulation of the transcripts of glutathion-S-transferase (GST), defensin (PDF), and catalase (CAT) following treatments with benzothiadiazole (BTH), dichloroisonicotinic acid (INA), or beta-aminobutyric acid (BABA) and/or *P. halstedii* inoculation. Preliminary results revealed significant increases in the activity of these genes in the susceptible but not in the resistant sunflowers, whereas the results with the partially resistant (HLI-type) sunflower were contradictory (Körösi and Virányi 2008). Apart from the plant activators, mentioned above, Nandeeshkumar et al. (2008a) reported about an enhanced and early activation of defence-related responses to downy mildew in a susceptible sunflower cultivar treated with chitosan. Using Northern hybridization analysis they revealed an increased level of transcripts for five known defence response genes. Furthermore, in a separate experiment, they also gained positive results with plant growth promoting rhizobacteria that were associated with an enhanced activation of catalase, phenylalanine ammonia lyase, peroxidase, polyphenoloxydase and chitinase (NandeeshKumar et al. 2008b). In addition, essential oil of a plant species *Bupluerum gibraltarium* was assumed to act as an

activator of plant defence in sunflower attacked by *P. halstedii* (Fernandez-Ocana et al. 2004). Perazzolli et al. (2008) demonstrated that besides benzothiadiazole, *Trichoderma harzianum* T39 strain effectively protected susceptible grapevine cultivars against downy mildew (*Plasmopara viticola*) under greenhouse conditions. Although no such experiments have yet been performed for the sunflower–*P. halstedii* pathosystem, the results with grapevine downy mildew might be of interest. They found differences between the two biotic and chemical inducers in terms of their activation time, persistence of the effect and systemic protection, suggesting that different pathways other than that of salicylic-acid dependent BTH elicitation are activated by *T. harzianum* in grapevine. It is still worth mentioning that plant activators (chemical resistance inducers) usually do not cause any retardation in plant growth or development of the treated crop plant but such unexpected side-effects might occur under specific conditions and/or with specific pathogen–host relations (LaMondia 2008).

Epidemiology and disease management

Disease incidence and severity, population dynamics

The incidence and severity of downy mildewed sunflower plants in the field has been reported by earlier investigators to range between traces to near 50% or even up to 80%, primarily depending on inoculum potential, the cultivar used, and the environmental conditions (for reference see Sackston 1981). Additionally, the time of evaluation can be crucial for estimating the degree of infection severity, as Gulya (2006) reported from the 2005 North Dakota (USA) sunflower crop situation. Based on field observations he concluded that late season surveys generally underestimate the impact of early season downy mildew, primarily because a portion of affected plants die early in the season and are not considered in autumn surveys. *Plasmopara halstedii* is present worldwide except for Australia where it has not been detected on sunflower so far. An up-to-date record of its global distribution was recently published by Gulya (2007) who listed as many as 35 pathotypes (virulence phenotypes). He found a considerable variation, by continent, on the number of pathotypes, Asia and South America having the fewest number of pathotypes identified followed by Africa, Europe and North America. The countries with the highest numbers of pathotypes are Canada, France and the USA. There could be various explanations for the appearance of new virulent forms (Delmotte et al. 2008), but the increase in pathotypes during the last decade is probably due to the introduction of new cultivars with different genetic pedigrees and to the intensity of production, particularly in Europe. Though it is known that local spread of this pathogen within a field is mainly by soil particles (e.g. during tillage) and windborne sporangia from adjacent infected sunflower plants, its introduction into new countries and continents can only be by seed.

Factors affecting disease development

P. halstedii as an oomycete producing motile zoospores, is considered to exclusively depend on the presence of free water to complete its life cycle. In this context, the most susceptible stage of host development is between germination and emergence (Meliala et al. 2000). As an example, severe downy mildew outbreaks were observed in 2005 in some North Dakota fields where unusually high amounts of precipitation occurred during June, immediately following planting (Gulya 2006). Similarly, Tourvieille et al. (2008a) investigated the incidence of spring rainfall on the severity of primary downy mildew attack of sunflower in field trials with staggered sowing dates. Disease risk appeared greatest if there was heavy rainfall when sunflower seedlings were at their most susceptible stage, whereas heavy rainfall before sowing or after emergence had no effect on the percentage of diseased plants. Furthermore, Göre (2009) recorded a serious outbreak of sunflower downy mildew in Turkey during the spring of 2007 and 2008. He thought that low temperature and extensive spring rains encouraged the disease, resulting in approximately 85% yield loss and lower quality of sunflower production in the Marmara region of Trace. Interestingly, Baldini et al. (2006), aiming at developing a prediction model, studied the main climatic factors affecting development and spread of downy mildew in high-oleic sunflower. By calculating the accumulated rainfall and average temperatures for different sowing date conditions, they did not find water availability as a limiting factor between accumulated rainfall and percentage of infection. In contrast, air temperature clearly had an effect on the percentage of

disease, the most favourable mean air temperatures being between 10–15°C during the 5 days after sowing.

Besides environmental conditions, disease intensity may also be influenced by the aggressiveness of the pathogen population. Recently, French researchers measured this important trait of *P. halstedii* by using pathotypes 100 and 710, as well as two sunflower lines differing in their level of quantitative resistance (Sakr et al. 2009). Based on two criteria, i.e., the latent period and sporulation density, they successfully differentiated between the two pathogen strains in terms of their aggressiveness.

Soilborne vs. airborne inoculum

Apart from the soilborne nature of *P. halstedii,* since oospores of this pathogen give rise to systemically infected plants (Spring and Zipper 2000), local lesions on leaves as a result of infection by wind-borne sporangia may occasionally occur in some areas. The first report of a massive appearance of this disease symptom was given as early as the 1970s by Zimmer (1972) in the United States, whereas in Europe such secondary local infections could hardly be seen until the late1990s. In a field survey in 1995 in Hungary, an unusual disease appearance was observed: numerous plants with angular leaf spots, some of which were located close to the main veins or near the end of petiole, were observed (Rátainé Vida 1996). From such leaves the pathogen tended to spread along the veins reaching the petiole. Subsequent microscopic observations gave evidence of a close correlation between the presence of hyphae in leaf and corresponding petiole (Virányi, unpublished). In fact, such secondary systemic spread of the pathogen has become more common in different sunflower cultivars under extremely favourable (wet and cool) weather conditions. Similar to Hungary, a high potential for transition from local to systemic infection was found by Spring (2008) in Germany as well. Though localized lesions are usually not considered to be of economic importance, in the case of seed production, seeds from such plants may carry-over the pathogen to the next season and to various locations (Spring 2008).

Detection, risk assessment

Considering the risk that seed transmission of *P. halstedii* poses to unexpected propagation of new pathogenic variants or fungicide-tolerant strains into other regions, it is necessary to guarantee the absence of the pathogen in seed shipments. With the rapid improvement of detection technology, it became more probable to find a method that could be sufficient and productive in monitoring sunflower seed samples for latent infection. Various methods have been used to develop diagnostic tools for the identification of this pathogen in sunflower plant tissue and/or seed samples. Roeckel-Drevet et al. (1999) generated an oligonucleotide primer for the selective amplification of *P. halstedii,* Bouterige et al. (2000) employed an enzyme linked immunosorbent assay (ELISA) test, and Spring and Haas (2004) were successful with fatty acid patterns as markers of infected vs. healthy sunflower seeds. Recently, Ioos et al. (2007) developed a method for the direct detection of the pathogen in seed samples based on the ribosomal large sub unit DNA. Though their test was able to detect a near 1:50 ratio of seed contamination, this was not practical, probably because of the problem of representative sampling. Furthermore, from the practical point of view it might be of interest for sunflower growers to know in advance, i.e. before sowing what pathotype (s) exist(s) and to what extent in a particular field. For this reason, Gulya (2004) and Tourvieille et al. (2008b) developed a bioassay using soil samples to assess downy mildew risk at the field level. Under different geographical and climatic conditions, their results revealed a good correlation between the soil infestation measured by the soil bioassay and the presence of infected plants in previous years.

Chemical control

The possibility of effective fungicidal control against sunflower downy mildew is closely associated with the introduction and use of metalaxyl in the late 1970s. This systemic, phenylamide-type compound was considered to be very effective against downy mildew of sunflower for almost two decades. However, the first tolerant strains could be identified in Hungary (Oros and Virányi 1984) and in Turkey (Delen et al. 1985), and expanded field populations tolerant to this fungicide have been described from France (Lafon et al. 1996), the United States (Gulya et al. 1999), Spain (Molinero-Ruiz et al. 2000) and Germany (Spring et al. (2006). The widespread occurrence of *P. halstedii* populations insensitive to metalaxyl/mefe-

noxam (the latter being a pure stereoisomer of the former) prompted researchers to look for alternative compounds in order to combat such fungicide tolerance. For example, in the United States, as many as 30 fungicides have been tested as seed treatment for the efficacy against sunflower downy mildew and even the most effective compound, fenamidone, allowed 3–16 % infection in field trials (Gulya 2002b). No report is available so far for the authors regarding the appearance of *P. halstedii* tolerance to fungicides other than phenylamides.

Outlook

In this review we tried to outline the recent progress in research and development of sunflower downy mildew involving various aspects of the pathogen's taxonomy and biology, host resistance, and the interaction between host and pathogen. Although our aim was to give an account of the latest experimental results published following the review by Gulya and co-workers in 1997, we often realized that this time-limit might hinder our general philosophy: besides providing an up-to-date and sufficient information package of the progress achieved in downy mildew research over the last decade, the continuous character of research activity was kept in mind as well. This makes it necessary to enhance some former aspects and to re-evaluate previous results in light of new experimental possibilities, particularly as contributed by molecular genetic techniques.

By reading through a number of scientific reports in journals and other periodicals we also realized that questions remained unanswered and some new questions arised. For example, future studies should 1) elucidate the final taxonomic status and the exact host range of this oomycete pathogen, and 2) clarify the origin of genetic variation within the species pathogenic on sunflower, and the local/non-local appearance of virulence phenotypes. Furthermore, until a reliable molecular method of pathotype identification is firmly established, the traditional methodology based on the interpretation of reactions on sunflower differential lines should be improved and the number of differentials possibly expanded. This includes the establishment of a valid system for the cultivation and distribution of seed samples required for pathotyping new isolates. New sources of resistance to downy mildew, including non-race-specific or durable resistance, need to be identified and special attention should also be paid to alternative types of resistance such as the use of abiotic/biotic resistance inducers. Finally, continuing use of chemical control will be required, but its efficacy should be improved by the discovery of new molecules and/or mixtures of various compounds in order to combat fungicide tolerance.

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