HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

PATHOTYPE COMPOSITION AND MEFENOXAM SENSITIVITY OF *PLASMOPARA HALSTEDII (FARLOW) BERLESE & DE TONI* (SUNFLOWER DOWNY MILDEW) IN HUNGARY AND THE POTENTIAL USE OF A BOTANICAL PESTICIDE IN THE MANAGEMENT

DOI: 10.54598/002940

PhD Dissertation

Nisha Nisha

Gödöllő

2022

The PhD School

Name: Doctoral School of Biological Sciences

Discipline: Agricultural and environmental microbiology

Head: Prof. Dr. Zoltán Nagy, DSc
Head of the Biological Doctoral School
MATE, Institute of Agronomy

Supervisor(s): Dr. habil. Rita Bán, PhD
Associate Professor,
MATE, Plant Protection Institute
Department of Integrated Plant Protection

....	…...
Approval of the Head of Doctoral School	**Approval of the Supervisor**

58 # Table of contents

59 1.INTRODUCTION ... 5
60 2.LITERATURE REVIEW ... 7
61 2.1 Significance of sunflower ... 7
62 2.2 History of *Plasmopara halstedii* ... 8
63 2.3 Significance of sunflower downy mildew and main characteristics of the pathogen ... 8
64 2.4 The high variability of *Plasmopara halstedii* – evolution and spread of pathotypes ... 10
65 2.5 International Standardised Nomenclature System for pathotype identification of *P. halstedii* ... 11
66 2.6 Symptoms of sunflower downy mildew ... 11
67 2.7 Life cycle of *Plasmopara halstedii* ... 12
68 2.8 Management of *Plasmopara halstedii* ... 14
69 2.8.1 Integrated pest management (IPM) against *Plasmopara halstedii* ... 14
70 2.8.2 Agricultural methods ... 14
71 2.8.3 Genetic control, types of resistance to sunflower downy mildew ... 15
72 2.8.4 Chemical control ... 17
73 2.9 Induced resistance ... 18
74 2.10 Fungicide resistance ... 21
75 2.10.1 Fungicide resistance of sunflower downy mildew ... 22
76 3. MATERIALS AND METHODS ... 23
77 3.1 Pathotype identification of *Plasmopara halstedii* isolates collected between 2017 and 2019 ... 23
78 3.1.1 Collection of diseased plant materials ... 23
79 3.1.2 Propagation of inoculum using whole seedling immersion (WSI) method ... 25
80 3.1.3 Characterization of *P. halstedii* pathotypes ... 26
81 3.2 Fungicide sensitivity tests of *Plasmopara halstedii* isolates ... 27
82 3.2.1 Fungicide sensitivity test performed with 10 *P. halstedii* isolates by using WSI method ... 27
83 3.2.2 Fungicide sensitivity test performed with 8 *P. halstedii* isolates by using soil drench inoculation
84 (SDI) method ... 29
85 3.2.3 Assessing the effect of different concentrations of mefenoxam on 5 *P. halstedii* isolates ... 31
86 3.3 Efficacy of neem-derived pesticides to restrict sunflower downy mildew ... 32
87 3.3.1 *In-vitro* experiment: Examination of the effect of neem-derived pesticides on *P. halstedii* sporangial
88 germination ... 32
89 3.3.2 *In-vivo* experiment: Effect of neem-derived pesticides on *P. halstedii* isolates (Mád and
90 Rákóczifalva) in sunflower ... 33
91 4.1 Pathotype identification of *P. halstedii* isolates collected in Hungary (2017-2019) ... 35
92 4.1.1 Identification of a new pathotype, 734, in Hungary ... 36
93 4.2 Fungicide sensitivity tests ... 37
94 4.2.1 Fungicide sensitivity tests performed on 10 *P. halstedii* isolates by using the whole seedling
95 immersion (WSI) method ... 37

96 4.2.2 Fungicide sensitivity tests performed on 8 *P. halstedii* isolates by using the soil drench inoculation
97 method 39
98 4.2.3 Effect of different concentrations of mefenoxam on 5 *P. halstedii* isolates in sunflower....................47
99 4.3 Effects of neem-derived pesticides on sunflower downy mildew 53
100 4.3.1 *In-vitro* experiment: Examination of the effect of neem-derived pesticides on *P. halstedii* sporangial
101 germination 53
102 4.3.2 Pre-treatment effect of neem-derived pesticides on *Plasmopara halstedii* isolates in *in-vivo*
103 conditions 54
104 5. DISCUSSION 58
105 5.1 Pathotype composition of sunflower downy mildew (*Plasmopara halstedii*) in Hungary.................... 58
106 5.2 Mefenoxam-sensitivity of Hungarian *P. halstedii* isolates 60
107 5.3 Efficacy of neem-derived pesticides to restrict sunflower downy mildew 63
108 6. CONCLUSIONS AND RECOMMENDATIONS 65
109 7. NEW SCIENTIFIC RESULTS.................... 66
110 8. SUMMARY 67
111 9. ACKNOWLEDGEMENTS 68
112 10. REFERENCES 70
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in the world. There are several abiotic and biotic environmental factors that negatively influence the yield of sunflowers and ultimately reduce oil production (Rauf 2019). Among these biotic environmental factors, *Plasmopara halstedii* (Farlow) Berlese et de Toni, the causal agent of sunflower downy mildew, infects preferably the sunflowers worldwide (Friskop et al. 2009, Sedlářová et al. 2013) and leads to crop loss of up to 85% (Ioos et al. 2007). This pathogen not only leads the crop loss but also enhances the cost of protection and resistance breeding in sunflower plants. Therefore, it has become necessary to study this pathogen against the sunflower plants and fulfill the utmost demands of oil to the growing population around the globe.

This disease is mostly initiated by the soil-borne oospores and occasionally from infected seeds. *Plasmopara halstedii* infection in the sunflower usually takes place in the below ground plant parts by direct penetration in the roots (Virányi and Spring 2011). The pathogen mainly infects seedlings via their roots by zoospores leading to systemic infection but sometimes may cause local foliar lesions by airborne sporangia. Root infection leads to seedling damping-off, or severe other symptoms, such as stunted plants (dwarfing), chlorosis of leaves, and white sporulation, which subsequently resulting in yield losses caused by the production of infertile flowers (Gascuel et al. 2015). Yield losses from downy mildew can be substantial, depending on the percentage of diseased plants across the field (Virányi and Spring, 2011).

Plasmopara halstedii rapidly develops races (pathotypes) that can break down the resistance genes in sunflowers (Sedlářová et al. 2016, Bán et al. 2018). *Plasmopara halstedii* is a highly variable and adaptive pathogen, which has about 50 pathotypes in the world nowadays (Spring et al. 2018, Spring 2019, Bán et al. 2021). The high variability of the pathogen significantly makes it difficult the effective disease management in sunflower cultivation. Thus, regular monitoring of the pathotype composition in a region or country is essential.

Downy mildew of sunflower can be controlled by using resistant cultivars carrying dominant *Pl* genes, agrotechnical methods, and chemical treatment (with fungicides) of the seeds with metalaxyl (Albourie et al. 1998). Metalaxyl is a phenylamide fungicide which provides systemic protection against oomycetes. Mefenoxam (the stereoisomer of metalaxyl) has been widely used for downy mildew control as a seed dressing since 1977 (Melero-Vara et al. 1982, Patil et al. 1991, Schwinn and Margot 1991). This active substance has been extensively applied to control many different oomycetes, including *P. halstedii*, *Phytophthora infestans* (Mont.) de Bary, *Peronospora tabacina* de Bary and *Bremia lactucae* Regel (Schwinn and Staub 1987, Mouzeyar et al. 1995). However, *P. halstedii* has developed resistance against this active ingredient in many countries

166 (Gascuel et al. 2015). To date, there is little or no data available in Hungary on the sensitivity of
167 the pathogen to mefenoxam. In addition, very little is known about plant responses in plants
168 infected with mefenoxam tolerant/resistant *P. halstedii* isolates.
169 Due to the high variability of the pathogen, traditional control methods need to be complemented
170 by new approaches based on the principles of integrated pest management which are sustainable
171 and economical. As a future alternative to fungicide treatments, efforts were made to control
172 disease via induced resistance and biological antagonism (Sackston et al. 1992). There have been
173 studies to test the effects of a botanical pesticide, neem (*Azadirachta indica* A. Juss), against
174 different pests. Neem-based plant protection products are known to possess antifeedant, antifungal
175 (Schmutterer 1988, Girish and Bhat 2008), nematicidal, insecticidal properties (Girish and Bhat
176 2008). There is preliminary (positive) data about neem's effect against sunflower downy mildew
177 (Doshi et al. 2020), so more intensive research is needed in this area before its widespread use in
178 the fields.

179 In view of the above, I have set the following objectives for my work:

180 ➢ Pathotype identification of *P. halstedii* (sunflower downy mildew) isolates collected from
181 different regions in Hungary in three consecutive years (2017-2019)
182 ➢ Testing the mefenoxam sensitivity of *P. halstedii* isolates collected in Hungary and
183 characterize host tissue responses to tolerant/resistant isolates with fluorescence
184 microscope
185 ➢ Investigations on the effectiveness of neem-derived pesticides on *P. halstedii* in sunflower
186 under *in-vitro* and *in-vivo* conditions
187

2. LITERATURE REVIEW

2.1 Significance of sunflower

Sunflower was introduced to Europe from North and Central America by Spanish explorers. The interest of sunflower for oil extraction was found in Russia. By the end of the 19th century, it had become an agricultural crop and began to be bred. Sunflower is one of the essential seed oil crops in the world. Cultivated sunflower (*Helianthus annuus* L.) is an annual diploid plant (2n=2x=34) and originated from North America. The genus *Helianthus* comprises 53 wild species from which 14 species are annual diploid (2n=2x=34) and 39 species are perennial, including 29 species diploid (2n=2x=34), 4 tetraploids (2n=4x=68) and 6 hexaploids (2n=6x=102) (Moyers and Rieseberg, 2013, Seiler and Jan 2014, Qi et al. 2016). Sunflower is the fourth most cultivated oilseed crop globally, right after oil palm, soybean and rapeseed but second in the European Union. Sunflower produces healthy oil rich in unsaturated fatty acids, and high content of vitamin E. Sunflower plant can cultivate under low water input regimes to compare other oil crops due to their higher adaptability and versatility (Kaya et al. 2012, Gascuel et al. 2015). The most susceptible stages for host plant development are germination and emergence of seeds (Meliala et al. 2000, Virányi and Spring 2011).

Europe is one of the biggest producers of sunflowers and is primarily cultivated in the Southern and Eastern regions. Globally, the most prominent leading producer is Russia, and other producing countries are Ukraine, the USA, Argentina, India, China, Turkey and South Africa (FAO). Among the major sunflower crop producing countries, Russia was the largest cultivator of sunflower crop in terms of harvested area, followed by Ukraine and Argentina and contributed about 56 percent of the total harvested area (FAO). Plant breeders increased the oil content, making it one of the most popular oilseed crops for consumers in the first half of the previous century. However, both (a)biotic stresses are significant constraints for sunflower production worldwide (Rauf 2019).

Sunflower is prone to be attacked by several pests and diseases, resulting in significant yield losses and poor quality of crop production. Diseases are the most significant limiting factor in sunflower production worldwide. Different diseases are dominant in different regions, depending on the prevailing environmental conditions. More than 30 different species of pathogens that attack sunflowers and cause economic loss in production have been identified so far. The most serious ones for the production of oil and confectionery sunflower are downy mildew (*Plasmopara halstedii*), Phomopsis stem cancer (*Diaporthe helianthi*), Sclerotinia stalk and head rot (*Sclerotinia sclerotiorum*), Charcoal rot (*Macrophomina phaseolina*), Verticillium wilt (*Verticillium dahliae*),

221 Rust (*Puccinia helianthi*), Phoma black stem (*Phoma macdonaldii*), Alternaria (*Alternaria* spp.)
222 and Rhizopus head rot (*Rhizopus* spp.) (Kaya et al. 2012).

223 2.2 History of *Plasmopara halstedii*

224 First time, it was reported by Halsted in 1876 from *Eupatorium purpureum* near the Bussay
225 Institution. Later, Farlow (1883) described the pathogen as *Peronospora halstedii* based on
226 samples found on *E. purpureum, Ambrosia artemisiifolia, Bidens frondosa, Rudbeckia laciniata,*
227 *Silphium terebinthaceum* and the perennial sunflower species *Helianthus strumosus, H. tuberosus*
228 and *H. doronicoides (H. mollis X giganteus).* Schröter (1886) had separated *Plasmopara* from
229 *Peronospora* due to the germination by means of zoospores and instead of forming germ tubes. In
230 1888, Berlese and co-author de Toni renamed *Peronospora* to the new genus under the name
231 *Plasmopara halstedii* (Farl.) Berl. et de Toni*,* and this name has been generally accepted
232 worldwide (Virányi and Spring 2011). Since then, numerous collections of downy mildew
233 collected on species of Asteraceae and classified as *Plasmopara halstedii* due to morphological
234 similarities of sporangiophores and sporangia. Moreover, Stevens (1913) reported high variability
235 in size and form of *P. halstedii* zoosporangia, which has become one of the most distributed
236 pathogens worldwide. In the middle of the 20th century, this disease caused by *P. halstedii*
237 expanded throughout Europe (Novotelnova 1966). According to Novotelnova's (1966)
238 observation, the pathogen showed geographical dissimilarities varied from Europe to North
239 America. However, Novotelnova renamed *P. halstedii* as *P. helianthi* but could not get acceptance
240 so far (Virányi and Spring 2011).

241 2.3 Significance of sunflower downy mildew and main characteristics of the pathogen

242 Sunflower downy mildew is caused by the plant pathogen *P. halstedii* (Farlow) Berlese et de Toni
243 and is one of the most serious diseases affecting sunflower production worldwide. This pathogen
244 had been under quarantine regulation in the European Union since 1992 (Delmotte et al 2008), and
245 more recently it is designated as a regulated non-quarantine pest (RNQP) (EPPO).

246 *Plasmopara halstedii* is a *Peronosporaceae*-family obligate biotrophic oomycete that requires a
247 living host to complete its life cycle (Fawke et al. 2015). Haustoria and mycelium help to uptake
248 nutrients from their hosts and release enzymes and effectors into the host's cells. In the absence of
249 resistant cultivars of sunflower and chemical control, it can cause complete loss of sunflower crop
250 and decline yield production. This pathogen is diploid, homothallic and reproduce via both
251 asexually and sexually.

252 Phytopathogenic oomycetes are different from fungi, including the hemibiotroph genus
253 *Phytophthora*, which causes late blight and obligate biotrophs, which causes downy mildew,

254 including the genera *Bremia*, *Peronospora*, *Plasmopara*. For downy mildew, the pathosystems
255 *Hyaloperonospora arabidopsidis/Arabidopsis thaliana* and *Bremia lactucae/ Lactuca sativa* are
256 well-studied model systems (Fawke et al. 2015).

257 *Plasmopara halstedii* is native to North America, and later reported in Russia and Western Europe
258 around 1960, where it was introduced probably through infected sunflower seeds. This pathogen
259 can be dispersed via wind, infected seeds, but mostly soil-borne (Ioos et al. 2007). The soil-borne
260 pathogen infects seedlings via penetrating underground host tissues, and systemic infection
261 follows. Under favourable conditions, a large number of zoosporangia are produced from
262 oospores, which release motile zoospores which are responsible for leaf infections on
263 neighbouring plants. Sakr et al. (2008), concluded that morphological characteristics of
264 zoosporangia are influenced not only by pathogen genetics but also on growth conditions such as
265 duration of incubation, infected plant parts, and, most importantly, the genotype of the host plant
266 (Sakr et al. 2008).

267 Mainly primary infections via roots and early secondary infections caused relatively higher yield
268 losses (Allard 1978), resulting in systemic infection of plants by the pathogen (Regnault and
269 Tourvieille 1991, Albourie et al. 1998). Secondary infections on the above-ground parts of
270 sunflower occur via the dispersion of zoosporangia. However, this type of infection slightly affects
271 the sunflower production economically but does not affect the yield significantly; mostly, such
272 infections remain local and temporary (Gulya et al. 1997, Spring 2009). According to Tourvieille
273 et al. (2008), heavy rainfall during the most vulnerable period of sunflower seedlings poses the
274 greatest risk of downy mildew.

275 It is considered that long-distance spreading of sunflower downy mildew might occur through the
276 exchange of oospore-contaminated seeds (Spring 2001). With the implementation of
277 contamination testing, there are at least possibilities to prevent the introduction of *P. halstedii* and
278 associated phenotypes (Virányi and Spring 2011, Spring 2019). Concerning that *P. halstedii* is
279 characterized by a high level of evolutionary potential (Sakr 2011b, 2012, Virányi and spring
280 2011), there have been several studies on virulence (Delmotte et al. 2008, Sakr 2011b, 2012,
281 Tourvieille et al. 2000, 2010) and more recently on aggressiveness (Sakr 2011a, b, c, 2012, Sakr
282 et al. 2011, Sakr 2013, Spring 2019, Bán et al. 2021).

283 Downy mildew has become a major threat to the sunflower crop because of the emergence of new
284 pathotypes and capable of infecting a variable range of sunflower genotypes. Thus, new pathotypes
285 of *P. halstedii* are bypassing sunflower hybrids resistance (Tourvieille 2000, Bán et al. 2021).

Therefore, diversification of resistant sources is a major objective of disease-resistant breeding (Rauf 2019).

2.4 The high variability of *Plasmopara halstedii* – evolution and spread of pathotypes

There are several pathotypes (races or virulence phenotypes) of *P. halstedii*, each with varying degrees of virulence. The widespread cultivation of sunflower hybrids with a rising number of developed resistance genes against *P. halstedii*, which induce genetic changes in the pathogen (Gascuel et al. 2015), is the cause of this high variability. In addition to mutation and sexual recombination, parasexual recombination provides an opportunity for genetic exchange between different pathotypes (Spring and Zipper 2006, Ahmed et al. 2012).

The number of pathotypes is constantly increasing around the world and even accelerated in the past decade. Most recently, 50 different pathotypes of *P. halstedii* had been identified worldwide (Spring 2019, Gilley et al. 2020, Miranda-Fuentes et al. 2021, Bán et al. 2021). Virányi et al. (2015) reviewed the race composition of *P. halstedii* in Europe, as well as in North and South America. Before 1980, there were only two pathotypes of *P. halstedii*: one in Europe (European race) and another in Red River Valley of North America (Red River race). Since 1980, pathologists all around the world have discovered novel *P. halstedii* isolates and identified them into pathotypes (Virányi et al. 2011, Gascuel et al. 2015).

The pathogen has continued to change its virulence character due to the adoption of novel resistance genes in sunflower hybrids (Gulya 2007, Virányi et al. 2015). This pathogen articulated high virulence diversity, especially in the making of pathogenic pathotypes and the spreading of pathotypes that overcome the *Pl6* resistance gene of sunflower is progressing (Bán et al. 2014, Iwebor et al. 2016). Indeed, pathotypes infecting Ha335 containing *Pl6* gene against *P. halstedii* were found in French (304, 307, 314, 334, 704, 707, 714, 717, 774; reviewed by Virányi et al. (2015), Czechian (705, 715; Sedlářová et al. 2016), Hungarian (704, 714; Bán et al. 2014), and Russian (334; Iwebor et al. 2016) sunflower fields over several years. So due to pathogenic variability, the pathogen influences the growth of new sunflower's hybrids and ultimately crop yield loss (Trojanová et al. 2017).

According to Virányi et al. (2015), the highest pathogenic diversity of *P. halstedii* has been recorded in Canada, USA and France, between 2007 and 2013. In France, race 304 was the first to overcome *Pl* resistant genes in 2000. Recently, highly aggressive *P. halstedii* pathotypes have been reported in several areas of Europe, including pathotype 354 in Germany (Spring and Zipper 2018), pathotypes 724 and 734 in Hungary (Bán et al. 2018, Nisha et al. 2021), pathotype 705 in Spain (García-Carneros and Molinero-Ruiz 2017), and pathotypes 705 and 715 in the Czech

319 Republic (Sedlářová et al. 2016) (for more details review Bán et at. 2021). Pathotype 734 is already
320 widespread in the United States and Russia and is considered very aggressive, having been able to
321 infect hybrids with resistance genes Pl_6 and Pl_7 (Iwebor et al. 2018) and 714 in Italy, which
322 overcome the action of Pl_8 in the line RHA-340. The pathotype 714 has already been described in
323 the Czech Republic, France, Hungary and the United States (Virányi et al. 2015, Bán et al. 2014,
324 Martín-Sanz et al. 2020). Rozynek and Spring (2000) studied pathotypes of sunflower downy
325 mildew in southern Germany. They identified pathotypes 730, 710, 330, 310 and 300 in this region.
326 However, these pathotypes have already been identified in other areas of Europe. In Bulgaria, there
327 are five pathotypes, 300, 330, 700, 721 and 731 identified by Shindrova (2010). Of these, race 700
328 has the largest distribution area (in northern Bulgaria) and accounts for 46% of the downy mildew
329 population. Alizadeh and Rahmanpour (2005) identified a race as the predominant race of downy
330 mildew on sunflower, *P. halstedii*, for surveyed areas in Iran. Moreover, the identified pathotype
331 was physiologically different from pathotypes identified worldwide determined proposed by
332 Gulya et al. (1991). However, the use of newly introduced differential lines is necessary to ensure
333 the presence of different physiological pathotypes, proposed by Tourvielle et al. (2000).

334 **2.5 International Standardised Nomenclature System for pathotype identification of *P.***
335 ***halstedii***

336 Gulya et al. (1998) suggested using a triplet code system based on virulence patterns of *P. halstedii*
337 isolates because of the rising number of new pathotypes. Gulya and co-workers described that the
338 pathotype characterization of *P. halstedii* determined by universally accepted international
339 standardised nomenclature system based on sunflower differential lines by using a triplet set of
340 inbred lines containing different major resistance (*R*) genes called *Pl*, and necessitating the
341 identification of further and possibly more durable broad-spectrum resistances (Pecrix et al. 2019).
342 Isolates of *P. halstedii* collected from diseased plants in the field are designated as pathotypes
343 based on virulence profiles in a set of differential lines of sunflower carrying different major *Pl*
344 resistance genes (Gascuel et al. 2015). Susceptible or resistant plants are defined by disease
345 symptoms and mainly via sporulation on the leaves.

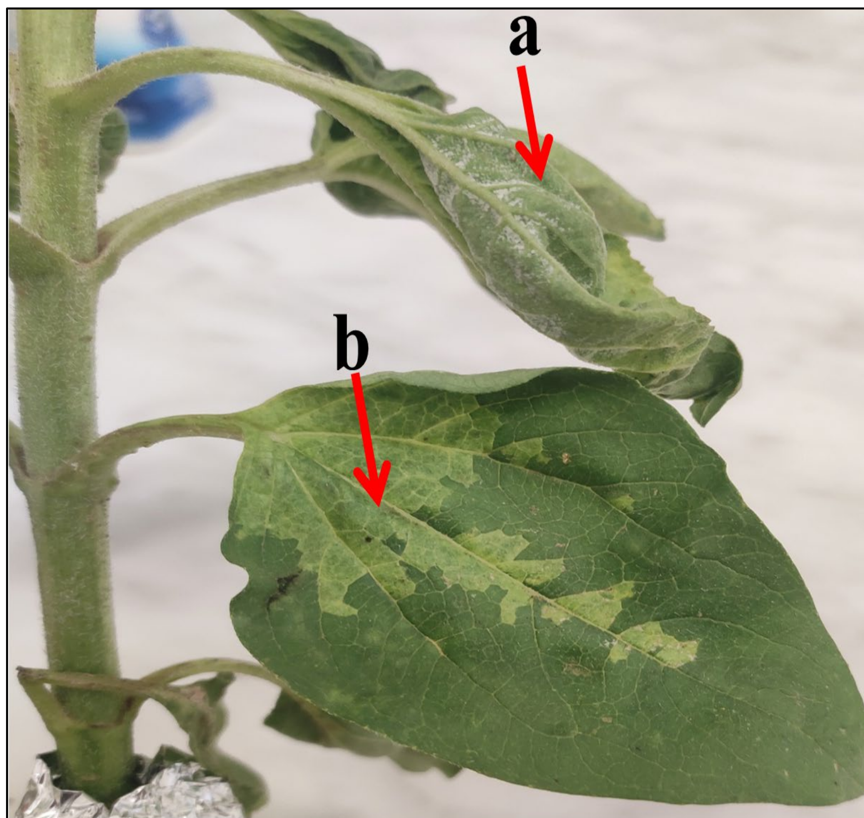
346 **2.6 Symptoms of sunflower downy mildew**

347 The symptoms of downy mildew on sunflower varies according to the age of tissue, the duration
348 of inoculum, cultivars used and the environment that influences the infection process (Spring
349 2001). In addition to environmental factors, the aggressiveness of the pathogen population also
350 influenced the disease intensity (Göre 2009).

351 Downy mildew causes white sporulation on the abaxial and adaxial sides of cotyledons, stunted
352 plants to varying degrees, pre- and post-damping-off, chlorosis in the leaves of affected plants,

353 which spreads along the main veins and over the lamella, and eventually leads to plant mortality
354 (Bán et al. 2017) (Figure 1). The cause of dwarfism is unclear; however, it may be due to hormonal
355 changes caused by nutrient-extracted nutrients (Gascuel et al. 2015). Dwarfing of diseased plants,
356 chlorosis along leaf veins, and small heads with sterile seeds are all symptoms of primary infection
357 followed by direct movement of zoospores toward the roots (Jocić et al. 2012, Gascuel et al. 2015).
358 Damping-off can occur as a result of a severe infection. Yield losses from downy mildew can be
359 substantial, depending on the percentage of infected plants and their distribution within the field
360 (Virányi 2008, Markell et al. 2015, Körösi et al. 2020).

361 Secondary infections by zoospores and sporangia that develop beneath the leaves have no impact
362 on disease spread or crop loss. Secondary infections can also become systemic, causing dwarfism
363 of affected plant parts (Spring 2009, Bán et al. 2021). In addition, secondary infection increases
364 the risk of the disease spreading latently through the seeds.



365 **Figure 1.** Signs and symptoms of sunflower downy mildew (a; sporulation, b; chlorosis)
366 (Source: N. Nisha 2022).

367 **2.7 Life cycle of *Plasmopara halstedii***

368 In oomycetes, sexual reproduction can be either homothallic or heterothallic. The pathogen uses
369 the sexual phase (oospores) for overwintering and the asexual phase (zoospores) for secondary
370 infection throughout the sunflower growth season (Gascuel et al. 2015). Overwintering oospores
371 are long-lived and can survive in soil up to 6-8 years (Sakr et al. 2009). Oospores germinate with

zoosporangia which release zoospores that are responsible for secondary infections (Tourvieille et al. 2000). It is considered that the aggressiveness of the pathogen evaluates on the quantity of zoosporangia (Sakr et al. 2008).

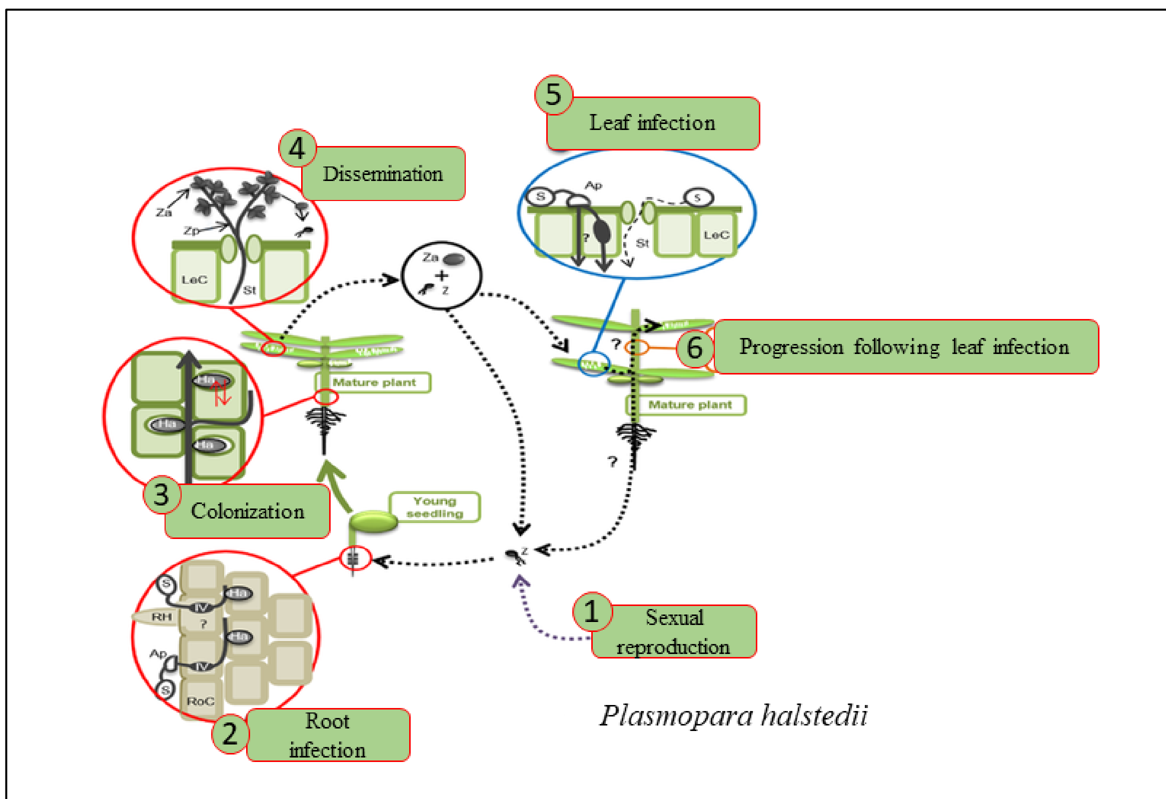


Figure 2. Life cycle of *Plasmopara halstedii.* (Source: Gascuel et al. 2015)

Generally, germination of zoospores (Z) from zoosporangia are produced by overwintering sexual oospores. Zoospores are freely motile asexual spores and flagellated. In the presence of soil free water, zoosporangia rapidly released zoospores and then occurs in contact with a sunflower root (Figure 2 (1)). Zoospores serve as the main source of inoculum after primary infection and germinate in a few hours. Zoospores have two modes of infection, either by direct penetration or indirect penetration. Direct penetration into a roots cell (RoC) with or without formation of an appressorium (Ap), pathogen increases osmotic pressure and enter into root epidermal cells and can be entered through injuries at the base of root hairs (RH) and formation of infection vesicles (IV) occurs (Figure 2 (2)). After penetration of pathogen into susceptible host tissue (compatible), it grows throughout the intercellular and intracellular between cortical cells and starts to colonise towards systematically shoot tissue, formed nutrition elements named haustoria/mycelium (Figure 2 (3)). Under favourable conditions (humidity and temperature), *P. halstedii* shows asexual reproduction structures by releasing zoosporangia (Za) from zoosporangiophores (Zp), and emerge on the lower sides of leaves and cotyledons via stomata (St) and below-ground tissues. Fully developed zoosporangia are the primary means of dissemination and infecting other plant leaves (Figure 2 (4)). Zoospores encyst around leaf trichomes and veins and start to germinate after

393 penetration into leaf tissues through intercellular spaces of parenchyma cells by making hyphae.
394 Penetration of zoospores via stomata is rarely observed (Figure 2 (5)). Pathogen progression in
395 plant tissues following leaf infections and is poorly characterized (Figure 2 (6)) (Gascuel et al.
396 2015). Oospores produced during sexual reproduction serve as primary inoculum for the next
397 season (Sakr et al. 2008).

398 **2.8 Management of *Plasmopara halstedii***

399 The pathogen has both asexual and sexual life cycle, thus making disease management difficult. It
400 is challenging to eradicate this pathogen once it established in an area. The situation is further
401 complicated by the variability of the pathogen, as more and more pathotypes appear year after year
402 and are able to infect the resistant hybrids. In general, therefore, the basic protective measures and
403 effective manner to manage sunflower downy mildew in sunflower is the use of integrated pest
404 management (Barzman et al. 2015).

405 **2.8.1 Integrated pest management (IPM) against *Plasmopara halstedii***

406 IPM is a sustainable approach for managing pests by combining and integrating all available
407 control measures, including monitoring, crop rotations, crop management and ecology, biological
408 control, mechanical and physical control, pesticide selection, etc., in a way that reduces human
409 health and environmental risks. IPM built on agronomic, mechanical, physical and biological
410 principles and suggested using selective pesticides only when other approaches do not work with
411 other tools (Barzman et al. 2015).

412 IPM acts in different forms that vary in time and space. It is shaped according to site-specific
413 factors such as regional cropping pattern, field size, type and availability of seminatural habitats,
414 the broader landscape, cultivation practices, pest pressure, R&D efforts, availability of training,
415 farmer attitude, and economics. More sustainable control strategies are needed due to negative
416 impacts of pesticides on human health and the environment, emerging pesticide resistance and
417 stricter regulations on pesticide residues in agricultural products (Spring et al. 2018).

418 **2.8.2 Agricultural methods**

419 Crop rotation is the most effective agronomic measures for pest control and has been used for
420 thousands of years. Crop rotation involves growing a sequence of crop species with the rotation of
421 different species on the same land to break the life cycle of the pathogens (Barzman et al. 2015).
422 Crop rotation practices increase yield and sustainable production. The reduced use of extended
423 rotations largely is due to the introduction of chemical fertilisers and pesticides. Ball et al. (2005)
424 addressed the effects of crop rotation on soil properties such as fertility, organic matter content,
425 water availability, soil structure, aggregation, bulk density and erodibility. Crop rotation is largely

426 ineffective due to the ability of oospore to survive in the soil for many years until conditions are
427 favourable for germination and infection (Gulya et al. 1997).

428 In addition to crop rotation, tillage methods, irrigation, weed and biological control have served
429 the purpose of controlling this pathogen. Weed management plays an essential role in disease
430 control because many weeds are host plants for the downy mildew. For example, a common
431 ragweed, *Ambrosia artemisiifolia* was first found to be infected by *P. halstedii* pathogen in
432 Hungary (Vajna 2002, Choi et al. 2009). Volunteer plants eradication is also important because
433 they can act as reservoirs for less and highly virulent pathogen variants (Bán et al. 2021). There
434 are few organism selected as antagonists of Pythium and other phytopathogenic oomycetes that
435 considered to be promising tools against *P. halstedii* due to their taxonomical proximity and
436 similar modes of action (Gulya et al. 1997).

437 **2.8.3 Genetic control, types of resistance to sunflower downy mildew**

438 Genetic resistance is the most effective, economic and environmentally friendly approach for
439 disease management and a sustainable strategy to increase crop yield and reduce fungicides use
440 (Mirzahosein-Tabrizi 2017, Qi et al. 2017).

441 Disease resistance of sunflowers to *P. halstedii* from plant breeding point of view can be divided
442 into two categories, as it is common for other diseases, as well. The first is qualitative resistance
443 which is mediated by the major *Pl* genes and tends to result in a disease-free plant. The second is
444 quantitative resistance which is controlled by minor genes and tends to affect the rate of disease
445 development (reducing the rate) rather than producing a disease-free plant (Tourvieille et al. 2008).
446 Genetic studies identified 36 major dominant *Pl* resistance genes (Pl_1–Pl_{35}, and Pl_{Arg},) up to 2019
447 (Ma et al. 2019). Downy mildew resistance genes (*R* genes) have been reported in sunflower and
448 wild species (Pl_1-Pl_{19}, Pl_{21}, Pl_{Arg}) so far, conferring resistance to at least one *P. halstedii* pathotype
449 (Ma et al. 2018). Fifteen of these genes Pl_1, Pl_2, Pl_5–Pl_8, Pl_{13}–Pl_{19}, Pl_{21}, Pl_{Arg} have been introduced
450 into specific linkage groups (LGs) of the cultivated sunflower genome (Kinman 1970, Fick and
451 Zimmer 1974, Miller and Gulya 1984, Miller and Gulya 1991, Seiler 1991, Mouzeyar et al. 1995,
452 Roeckel-Drevet et al. 1996, Vear et al. 1997, Bert et al. 2001, Molinero-Ruiz et al. 2003a, Yu et
453 al. 2003, Mulpuri et al. 2009, Bachlava et al. 2011, Vincourt 2012, Liu et al. 2012, Qi et al. 2015,
454 Qi et al. 2016, Zhang et al. 2017) , and conferring resistance to one or more pathotypes of *P.*
455 *halstedii* and three quantitative trait loci (QTL) associated with partial resistance of downy mildew
456 were identified on LGs 7, 8, and 10, respectively (Vear et al. 2008a, Vincourt et al. 2012, Qi et al.
457 2017). *Pl* genes originated mostly from wild *H. annuus* and other *Helianthus* species (*H.*
458 *argophyllus, H. praecox* and *H. tuberosus*) (Vear et al. 2008b, Gascuel et al. 2015). Introgressive
459 hybridisation with wild species is widely used to broaden the genetic base of cultivated sunflower

460 (Qi et al. 2016) shown in (Table 1). In addition, Tourvieille et al. (2010) reported that the life
461 expectancy of *Pl* gene seems to be very short (less than 10 years) which is due to the important
462 use of *Pl* gene under conditions of increased infection and various selection pressures (Sakr
463 2011b). In the last 40 years, several resistance genes against *P. halstedii* pathotypes have become
464 inefficient in sunflower (Ahmed et al. 2012). For instance, it was reported that the downy mildew
465 R genes Pl_6 and Pl_7 were overcome by new pathogen pathotypes in 2009-2010 in the United States
466 (Gulya et al. 2011). In Argentina, the widely used downy mildew R gene Pl_{15} has been overcome
467 since 2013 (Castaño 2018). Therefore, there is further need to research for the characterization of
468 resistances that will be effective against such pathotypes of *P. halstedii*.

469 **Table 2.** Resistance genes incorporated in sunflower against downy mildew

Resistance genes	Inbred lines	Linkage groups	References
Pl_1	RHA265, RHA266	LG8	Kinman 1970
Pl_2	RHA274	LG8	Fick and Zimmer,1974
Pl_5	DM-2	LG13	Miller and Gulya 1984
Pl_6	HA335, HA336	LG8	Miller and Gulya 1991
Pl_7	HA337, HA338, HA339	LG8	Miller and Gulya 1991
Pl_8	RHA340	LG13	Miller and Gulya 1991
Pl_{13}	HA-R5	LG1	Mulpuri et al. 2009
Pl_{14}	-	LG1	Bachlava et al. 2011
Pl_{15}	RNID	LG8	de Romano et al. 2010
Pl_{16}	HA-R4	LG1	Roeckel-Drevet et al. 1996, Liu et al. 2012
Pl_{17}	HA 458	LG4	Qi et al. 2015
Pl_{18}	HA-DM1	LG2	Qi et al. 2016
Pl_{19}	-	LG4	Zhang et al. 2017
Pl_{21}	PAZ2	LG13	Vincourt 2012
Pl_{Arg}	Arg1575-2	LG1	Seiler 1991

470

471 Two types of sunflower-*P. halstedii* incompatibility responses have previously been found,
472 depending on the host-pathotype combination. Mouzeyar et al. (1994) differentiate between
473 resistance type I and type II. Type I resistance can limit pathogen development to the roots and the
474 hypocotyl basal zone, however type II resistance cannot, allowing the infection to reach throughout

475 the whole hypocotyl and sporulates on cotyledons. Mouzeyar et al. (1993) proved that *P. halstedii*
476 may infect both susceptible and resistant sunflower lines in a microscopic examination.
477 Hypersensitivity-like reactions, necrosis formation, and cell-division are well known defence
478 reactions in incompatible combinations (Mouzeyar et al. 1993, 1994, Radwan et al. 2011) and
479 fungicide treatments (Mouzeyar et al. 1995). A hypersensitive response (HR) occurs in the
480 hypocotyls of both types I and II resistant plants five days after root infection, but the fate of the
481 infection is determined by both the resistance gene in the host and the avirulence (avr) gene in *P.*
482 *halstedii.* The pathogen is restricted to the basal part of the hypocotyls in plants with type I
483 resistance, but in plants with type II resistance, the pathogen can penetrate the hypocotyls fully
484 and reach the cotyledons, although this rarely reaches the true leaves (Mouzeyar et al. 1993, 1994).
485 Gulya et al. (1991) and Sackston (1992) were the first to describe this phenomenon, which is
486 known as Cotyledon Limited Infection (CLI), which is a kind of Type II resistance (Radwan 2011).
487 In incompatible plant-pathogen interactions, recognition of a potential pathogen often leads to a
488 hypersensitive reaction (HR), with programmed cell death (PCD) activated at the site of attack to
489 halt the spread of the pathogen. During a HR, a small group of cells in the vicinity of the pathogen
490 undergo rapid PCD, usually within 12–24 hours of inoculation (Hermanns et al. 2003, Radwan et
491 al. 2005). Heller et al. (1997) proposed several mechanisms for latent infection and showed that
492 plants with latent infections have increased cell division activity that prohibits the pathogen in the
493 pith parenchyma and that hypersensitive responses confined the pathogen to the cortical
494 parenchyma.

495 **2.8.4 Chemical control**

496 Seed treatments can be a very effective management tool because they are most active when
497 seedlings germinate, and systemic infection usually occurs within a short time after planting (3 to
498 15 days) (Gulya et al. 2013, Humann et al. 2016, Humann et al. 2019). Metalaxyl is a systemic
499 phenylalanine fungicide considered a fairly effective measure to control the downy mildew of
500 sunflower as seed dressing (Albourie et al. 1998) and provides systemic protection against
501 oomycete pathogens. The active enantiomer of the racemic fungicide metalaxyl was replaced with
502 mefenoxam. The fungicide is administered at frequencies similar to those used with metalaxyl but
503 at lower rates (Parra and Ristaino 2001). Despite severe resistance problems in the oomycetes,
504 mefenoxam, an active ingredient, has been used widely for control of different oomycete
505 pathogens, including *P. halstedii, Phytophthora infestans, Peronospora tabacina,* and *Bremia*
506 *lactucae*, because of its excellent preventive, curative and eradicated activities (Morton et al. 1988,
507 Parra and Ristaino 2001, Pintore et al. 2016).

508 Although reduced sensitivity to metalaxyl (tolerance or resistance to this compound) had already
509 been described in several oomycete fungi soon after the introduction of this chemical into the field,
510 *P. halstedii* retained its sensitivity until recently, except that such tolerant strains could be detected
511 under laboratory conditions (Oros and Virányi 1984).

512 Oxathiapiproline (OXA) is the first member of the piperidinyl thiazole isoxazoline class of
513 fungicides (FRAC 49) and was recently discovered and developed by DuPont Crop Protection
514 (Pasteris et al. 2016). OXA has been shown to be effective against economically important
515 oomycete pathogens in other crops (Ji et al. 2014, Kness et al. 2016, Patel et al. 2015). This
516 fungicide has a different mode of action than fungicide seed treatments currently available for
517 sunflower and its efficacy and flexibility in application suggest that OXA may be a useful tool for
518 downy mildew control (Humann et al. 2019).

519 The sensitivity of *P. halstedii* pathotypes to phenylamides needs to be continuously monitored,
520 and fungicides with different modes of action are needed in fields where resistance is observed
521 (Molinero-Ruiz et al. 2005).

522 **2.9 Induced resistance**

523 Active ingredients like metalaxyl or related compounds play a significant role in controlling the
524 disease. However, fungicides are not cost-effective and pose severe environmental hazards
525 (Barzman et al. 2015). Several abiotic and biotic agents have been reported that can induce plant
526 resistance to pathogens. As a future alternative to fungicide treatments, efforts were made to
527 control disease via chemically induced resistance and biological antagonism (Sackston et al. 1992).

528 Apart from genetic resistance, induced resistance has been considered an effective and long-lasting
529 method for plant disease management. Induced resistance is the activation of plant defence
530 mechanisms triggered by avirulent and virulent pathogens. In broad terms, induced resistance can
531 be divided into two main types: systemic acquired resistance (SAR) and induced systemic
532 resistance (ISR). It is understood that the term induced systemic resistance (ISR) is used to describe
533 resistance caused by non-pathogenic microorganisms, and natural or synthetic elicitors (Kuć 2001,
534 Vallad and Goodman 2004). Induced systemic resistance is a phenomenon that has been
535 extensively studied in many plant–pathogen interactions and is induced by localised infection or
536 by treatments with microbial components or products or by a diverse group of structurally
537 unrelated organic and inorganic compounds (Kuć 2001). In contrast, the term systemic acquired
538 resistance (SAR) is used to describe resistance that is activated after plant exposure to the pathogen
539 and provides protection through a series of induced proteins (Oostendorp et al. 2001, Durrant and
540 Dong 2004, Conrath 2006). The difference between ISR and SAR is that SAR is mediated by

541 salicylic acid (SA), whereas ISR is mediated by the jasmonic acid (JA) or ethylene pathway (ET).
542 There are many data indicating that plant growth regulators such as salicylates and jasmonates can
543 be used to control fungal diseases (Kepczynska and Kepczynska 2005, Hayat et al. 2010,
544 Kępczyńska and Król 2011). Induced resistance is also triggered by chemical inducers such as
545 salicylic acid (SA), 2,6-dichloroiso-nicotinic acid (INA), Jasmonic acid, Bion 50 WG (benzo
546 (1,2,3)-thiadiazole-7-carbothionic acid-S-methyl ester or acibenzolar-S-methyl, (ASM or BTH))
547 and DL- β -amino butyric acid (BABA) (Van Loon et al. 1998, Heil and Bostock 2002, Bán et al.
548 2004, Jayaraj et al. 2004, Vallad and Goodman 2004, Körösi et al. 2009, Körösi et al. 2011, Sillero
549 et al. 2012).

550 Induced resistance mediated by rhizobacteria has also been studied in various plant species against
551 different pathogens (NandeeshKumar et al. 2009). NandeeshKumar et al. (2008a) reported that
552 plant growth-promoting rhizobacteria (PGPR) strain INR7 induced resistance against *P. halstedii*
553 in sunflower was mediated through enhanced expression of defence mechanisms like catalase,
554 peroxidase, polyphenol oxidase, phenylalanine ammonialyase, and chitinase. Treatment with
555 PGPR strain INR7 effectively reduced the incidence of downy mildew in the sunflower plants in
556 a concentration-dependent manner, and treatment of sunflower seeds with 1×10^{8}cfu/mL of PGPR
557 strain INR7 reduced disease severity and provide 51% protection under greenhouse conditions and
558 54% in field conditions, respectively. PGPR bacteria could be a beneficial component of integrated
559 disease management (NandeeshKumar et al. 2008a).

560 Moreover, seed treatment with plant growth-promoting fungi (PGPF) resulted in improved disease
561 protection against the downy mildew in sunflower (Nagaraju et al. 2012a). Similarly, seed
562 treatment with PGPF, especially *Trichoderma harzianum*, was reported to improve seed and plant
563 growth parameters and induce systemic resistance in sunflower plants against the downy mildew
564 caused by *P. halstedii* (Nagaraju et al. 2012b). β-aminobutyric acid (BABA), a non-protein amino
565 acid, has been shown to induce resistance in plants against a range of microbial pathogens which
566 includes fungi, bacteria, oomycetes, nematodes, viruses and abiotic stresses (Jakab et al. 2001,
567 Conrath et al. 2002, Cohen 2002, Ton and Mauch-Mani 2004, Justyna and Ewa 2013). In addition,
568 it not only induces resistance to stress factors, but can also stimulate plant growth and development
569 (Justyna and Ewa 2013). BTH is a non-toxic synthetic chemical that has been identified as a potent
570 inducer of SAR in several crops (Serrano et al. 2007). Tosi et al. (1998) showed that BTH protected
571 susceptible sunflower plants from *P. halstedii* infection. Bán et al. (2004) also reported that
572 treatment with Bion 50 WG significantly reduced fungal sporulation and plant damped-off in
573 compatible host-pathogen interactions and induced resistance in sunflower. It has been found that
574 BABA and chitosan induced resistance against *P. halstedii* in sunflower was mediated via the

575 enhanced activation of genes for defence related proteins in susceptible sunflower seedlings
576 (NandeeshKumar et al. 2008a, 2009). Körösi et al. (2011) also reported that BTH, BABA and INA
577 induced systemic resistance against *P. halstedii* in sunflower. BABA also provided significant
578 control of the late blight pathogen *Phytophthora infestans* on tomato (Sharma et al. 2012), and
579 reduced severity of *P. viticola* infestation in grapevines by 62% in field experiments (Tamm et al.
580 2011). Interestingly, BABA-induced the protection of *Brassica napus* from the fungal pathogen
581 *Leptosphaeria maculans* was also associated with a combination of modes of action, as it induced
582 synthesis of SA and the expression of PR-1, but also exerted a direct fungitoxic effect against the
583 pathogen (Šašek et al. 2012). Neem-derived pesticides could be an alternative to chemical
584 pesticides. In addition to microorganisms, it has also been reported that plant extracts can induce
585 resistance in plants to a number of pathogens. For example, Bhuvaneswari et al. (2012) reported
586 the induction of systemic acquired resistance (SAR) in *Hordeum vulgare* to *Drechslera graminea*
587 by fruit extracts of *Azadirachta indica* Juss. (Neem) by increasing in the activities of phenylalanine
588 ammonia lyase (PAL) and tyrosine ammonia lyase (TAL). Induction of resistance by seed
589 treatment with acibenzolar-S-methyl and methyl jasmonate against *Didymella bryoniae* and
590 *Sclerotinia sclerotiorum* in melon with rapid increase in the activity of chitinase and peroxidase
591 proteins associated with pathogenesis (Buzi et al. 2004). Furthermore, essentials oils were
592 examined with different concentrations against *P. halstedii* and found to be effective to decrease
593 the sporangium quantity (Er et al. 2021). More recently, it was reported that neem-derived
594 pesticides, namely neem leaf extracts (NLE) and azadirachtin (NeemAzal T/S) protect against
595 downy mildew in sunflower's susceptible cultivars (Doshi et al. 2020). Neem is the most studied
596 plant because of its wide range of effects against various plant pests and pathogens (Biswas et al.
597 2002). For example, Hasan et al. (2005) studied the antifungal effects of neem along with other
598 plant extracts against seed-borne fungi of wheat seeds and reported that the alcoholic extracts of
599 neem completely controlled the growth of *Bipolaris sorokiniana* (Sacc.), *Fusarium* spp.,
600 *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. after the treatment on wheat seeds. There
601 have been only few studies of neem against different oomycetes. For instance, Rashid et al. (2004)
602 investigated neem leaf diffusate, neem leaf powder and neem seed cake against *Phytophthora*
603 *infestans* (Mont.) De Bary and found that neem was effective in controlling the infection.
604 Similarly, different neem products such as crude neem seed oil, crude neem seed oil terpenoid
605 extract, nimbokil and neem leaf decoction has been tested against *P. infestans* by Mirza et al.
606 (2000). The only study examining the effect of neem against *Plasmopara viticola* was conducted
607 by Achimu and Schlösser (1992) where they successfully controlled the pathogen *in vitro*
608 conditions. BTH (acibenzolar-S-methyl) was originally marketed to control powdery mildew in
609 wheat and barley in Europe (Görlach et al. 1996). ASM and INA are considered the best chemical

610 elicitors available for inducing resistance. They are considered functional analogues of SA and
611 cause a systemic form of induced resistance across a broad range of plant pathogens (Friedrich et
612 al. 1996, Maleck et al. 2000). These chemicals did not exhibit direct antimicrobial activity;
613 however, some cases of antimicrobial activity associated with high elicitor concentrations have
614 been reported (Tosi and Zazzerini 2000, Rohilla et al. 2002, Ghazanfar et al. 2011). For example,
615 ASM was shown to induce SAR in rust (*Uromyces viciaefabae*) and *ascochyta* blight (*Ascochyta*
616 *fabae*) on faba bean both in the glasshouse and under field conditions (Sillero et al. 2012). ASM
617 has also been reported to control rust infection, caused by *Uromyces pisi* on pea plants, although
618 again control was not complete (Barilli et al. 2010). In this case, ASM induced resistance was
619 associated with increased activity of defence-related enzymes and phenolic content, and indeed
620 there was evidence of activation of defence enzymes by ASM treatment of both susceptible and
621 resistant genotypes (Barilli et al. 2010, Walters et al. 2013).

622 **2.10 Fungicide resistance**

623 Fungicide resistance is a selection mode that describes a fungus's ability to survive and reproduce
624 in the presence of a fungicide and causes poor disease control. However, several key factors
625 influence an organism's susceptibility to fungicides: (i) the pathogen's biology, (ii) the fungicide's
626 mechanism(s) of action, and iii. the rate and frequency of fungicide treatment.

627 Fungicides are essential tools for preventing and managing plant disease in modern crop
628 production (Vincelli 2014). However, due to the repeated use of fungicides (especially
629 mefenoxam), some novel pathotypes have developed fungicide resistance and have overcome
630 plant genetic resistance. The frequent use of resistant host cultivars is a significant selective driver
631 of pathogen evolution in agro-ecosystems. Typically, a single resistant crop cultivar is widely
632 utilised until the pathogen overcomes its resistance, at which point it is replaced by another. In the
633 presence of significant host selection, this cycle of pathogen evolution is sometimes referred to as
634 the 'boom and bust cycle' (Thompson and Burdon 1992), and it has been described for a variety of
635 powdery mildews and cereal rusts (McDonald and Linde 2002).

636 The generation of novel virulence in crop pathogen systems is influenced by a number of factors
637 (Brasier 1995, Kaltz and Shykoff 1998). Because pathogens have a shorter generation time than
638 their hosts, they can evolve quickly and improve their local adaptation (Ahmed et al. 2012).
639 However, the evolution of novel pathogenicity is not entirely dependent on recombination, and
640 there are numerous striking examples of evolution through mutation accumulation. For example,
641 in highly clonal populations of wheat rusts, evolution viably mutation was sufficient to allow rapid
642 quick adaptation to resistant host cultivars in highly clonal populations of wheat rusts (Enjalbert
643 et al. 2005).

644 In the 1960s, a new generation of fungicides was developed, beginning with benzimidazoles. They
645 are highly active and exhibit low phytotoxicity due to their specific mode of action against a target
646 protein in fungal pathogens. The majority of these site-specific fungicides are systemic, which
647 means they can penetrate the cuticle and spread throughout the plant, increasing their activity.
648 Resistance development in pathogen populations and loss of fungicide activity were noticed within
649 a few years following the introduction of site-specific fungicides, with *Botrytis cinerea* being one
650 of the first fungi to develop resistance. Since then, the problem of resistance has gained more
651 attention, and it has become a major focus of fungicide research.

652 Resistance development is influenced by a number of factors, including the fungicide's chemistry
653 and mode of action, the biology and of the target fungus' biology and reproductive capabilities,
654 and the frequency with which the fungicide is applied (Brent and Hollomon 1998, Hahn 2014). It
655 is a concern for all pesticides, including fungicides, insecticides, and herbicides (Vincelli 2014).

656 **2.10.1 Fungicide resistance of sunflower downy mildew**

657 Field isolates tolerant to metalaxyl were found first in France (Lafon et al. 1996, Delos et al. 1997,
658 Albourie et al. 1998), then in the USA (Gulya et al. 1999), and something similar happened in
659 Spain (Molinero-Ruiz et al. 2003b) and Italy (Covarelli and Tosi 2006). Resistance of *P. halstedii*
660 to mefenoxam has also been reported in Russia (Iwebor et al. 2019, Iwebor et al. 2021).

661 However, no reduced sensitivity was found in Hungary, although the number of samples examined
662 so far does not allow saying with certainty that this phenomenon is lacking in our country. In the
663 1980s, Oros and Virányi (1984) demonstrated the presence of tolerant *P. halstedii* strains in
664 greenhouse experiments in Hungary, but could not prove this in further tests with field isolates
665 (Virányi and Walcz 2000). More recently, Körösi et al. (2020) reported the mefenoxam tolerance
666 of *P. halstedii* pathotypes in Hungary.

667 Sunflower downy mildew is almost worldwide (Spring 2019) and the rapid development of new
668 aggressive pathotypes makes chemical disease control inevitable (Virányi and Spring 2011, Spring
669 et al. 2018). Although variability/diversity of pathogen makes the disease control difficult and
670 develop tolerance to fungicides.

671 It is believed that the mode of action of metalaxyl is by the selective inhibition of ribosomal RNA
672 synthesis (Davidse et al. 1983, Fisher and Hayes 1984, Davidse 1995). RNA polymerase is the
673 target site for metalaxyl, and an alteration of this target site can lead to resistance in some oomycete
674 pathogens (Davidse et al. 1983, Parra and Ristaino 2001).

675

676 # 3. MATERIALS AND METHODS

677

678 ## 3.1 Pathotype identification of *Plasmopara halstedii* isolates collected between 2017 and 2019

679 ### 3.1.1 Collection of diseased plant materials

680 Infected leaves of different sunflower hybrids carrying the *Pl6* resistance gene against sunflower
681 downy mildew, were collected from different parts of Hungary between 2017 to 2019 (Table 2).
682 Collected samples of *P. halstedii* isolates were transferred to the lab (Department of Integrated
683 Plant Protection, Institute of Plant Protection, Hungarian University of Agriculture and Life
684 Sciences, Gödöllő, Hungary) and then stored at -70 °C in a deep freezer until use. A total of 22 *P.*
685 *halstedii* isolates were characterized during the experiments (Table 2) (Appendix 1).

686

687 **Table 2.** List of *Plasmopara halstedii* isolates collected from Hungary during 2017-2019

Isolate number	Isolate ID	Geographic origin	Year of collection	Sunflower genotype (*Pl* gene)
1	Ph-20170613-23/1-Hu	Karácsond (HU)	2017	*Pl*6
2	Ph-20170523-2/1-Hu	Martfű (HU)	2017	unknown
3	Ph-20170609-18/1-Hu	Galgahévíz (HU)	2017	*Pl*6
4	Ph-20170621-28/1-Hu	Csongrád (HU)	2017	*Pl*6
5	Ph-20170529-4/1-Hu	Hatvan (HU)	2017	volunteer
6	Ph-20170529-4/2-Hu	Hatvan (HU)	2017	volunteer
7	Ph-20170703-40/1-Hu	Pély (HU)	2017	unknown
8	Ph-20170613-22/1-Hu	Túrkeve (HU)	2017	*Pl*6

9	Ph-20170622-29/C1-Hu	Bonyhád (HU)	2017	*Pl*6
10	Ph-20170622-29/B-Hu	Bonyhád (HU)	2017	*Pl*6
11	Ph-20170606-15/B-Hu	Vésztő (HU)	2017	*Pl*6
12	Ph-20170628-31/1-Hu	Szeged (HU)	2017	Experimental line
13	Ph-20170601-12/1-Hu	Abony (HU)	2017	*Pl*6
14	Ph-20170530-7/1-Hu	Tápé (HU)	2017	*Pl*6
15	Ph-20170630-34/A-Hu	Szamoskér (HU)	2017	*Pl*6
16	Ph-20180601-4/1-Hu	unknown (HU)	2018	*Pl*6
17	Ph-20190522-7/3-Hu	Békésszentandrás (HU)	2019	*Pl*6
18	Ph-20190627-21/1-Hu	Léh (HU)	2019	*Pl*6
19	Ph-20190606-14/1-Hu	Bucsa (HU)	2019	*Pl*6
20	Ph-20190606-14/3-Hu	Kertészsziget (HU)	2019	*Pl*6
21	Ph-20190606-14/4-Hu	Kötegyán (HU)	2019	*Pl*6

22	Ph-20190618-18/2-Hu	Vanyarc (HU)	2019	*Pl*6

688

689 ### 3.1.2 Propagation of inoculum using whole seedling immersion (WSI) method

690 Iregi szürke csíkos (a Hungarian sunflower cultivar susceptible to all the pathotypes of *P. halstedii)*
691 was used for the propagation of pathogen inoculum. Seeds were surface sterilized in 1% NaOCl
692 for 3-5 min, then rinsed in running tap water and germinated between wet filter papers for three
693 days at 20 °C until radicles reached a length of 2 to 5 cm. The white zoosporangia from infected
694 field leaves were washed off into bidistilled water and this suspension was adjusted to a
695 concentration of 35000 sporangia per mL by Burker chamber. The whole seedling immersion
696 (WSI) method (Cohen and Sackston 1973, Körösi et al. 2021) was used for inoculation, i.e., the 3-
697 day old seedlings were incubated in a sporangial suspension at 16 °C in the dark for overnight
698 (Figure 3). The inoculated sunflower seedlings were sown in trays containing horticultural perlite
699 (d = 4 mm). The plants were grown in a growth chamber with a photoperiod of 12 h at 22 °C, light
700 irradiance of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Figure 3). The plants were watered regularly.

701

702 **Figure 3:** Plants growing in the growth chamber

703

704 Nine days after inoculation, the plants were sprayed with bidistilled water and covered by dark
705 plastic polyethylene bags overnight (at 19 °C) to induce sporulation (Figure 4). Collected
706 sporangia were used as the inoculum for the characterization of pathotypes.

707

708 **Figure 4:** Plants covered with polyethylene bag to induce sporulation in dark at 19 °C

709

710 ### 3.1.3 Characterization of *P. halstedii* pathotypes

711 The preparation of the seeds and inoculum, as well as the method of the inoculation and growing
712 of the plants, were the same as described in the chapter 3.1.2 (Propagation of inoculum using WSI
713 method). However, for the pathotypes characterization, seedlings were inoculated by the
714 concentration of 50 000 sporangia/mL and were sown in trays containing 15 seedlings per each
715 differential line. Plants were grown for 3 weeks.

716 The disease was evaluated firstly after sporulation, according to the white sporangial coating on
717 cotyledons, and secondly, based on damping-off, as well as according to the chlorosis on true
718 leaves of 21-day old plants. Reaction of plants was determined as susceptible (S) or resistant (R),
719 according to the results of second evaluation.

720 The pathotype identification of *P. halstedii* isolates was performed by the universally accepted
721 standardized nomenclature method as described by Trojanová et al. (2017) using the nine
722 sunflower differential inbred lines (cv. Iregi szürke csíkos or HA-304 (susceptible lines), RHA–
723 265, RHA-274, PMI-3, PM-17, 803-1, HAR-4, QHP2, and HA-335) all containing different *Pl*
724 resistance genes against *P. halstedii* (Table 3). A score for each differential line was determined
725 based on the reaction of the plants (S or R) and the location of the differential line inside the triplet:
726 1, 2, and 4 scores can be given for susceptible lines located in the first, second, and third place
727 inside the triplet, respectively. The pathotype code was determined as the sum of scores by each
728 triplet and results in a three-digit code (coded virulence formula, CVF) (Table 3). The CVF

729 provides information about the virulence pattern of the isolate. The test was repeated twice with
730 two repetitions by each.
731
732 **Table 3.** Sunflower differential lines used for pathotype identification for *P. halstedii* in the
733 experiment and resistance genes incorporated (based on Gascuel et al. 2015).

Nomenclature			
Triplet	**Score**	**Sunflower differential lines**	**Resistance gene to *P. halstedii***
1st	1	Iregi Szürke Csíkos	No *Pl* gene
	2	RHA-265	*Pl1*
	4	RHA-274	*Pl2/Pl21*
2nd	1	PMI-3	Pl_{PMI3}
	2	PM-17	*Pl5*
	4	803-1	*Pl5+*[b]
3rd	1	HAR-4	Pl_{15}
	2	QHP-2	$Pl1/Pl_{15}$
	4	HA-335	*Pl6*

734
735 **3.2 Fungicide sensitivity tests of *Plasmopara halstedii* isolates**
736 **3.2.1 Fungicide sensitivity test performed with 10 *P. halstedii* isolates by using WSI method**
737 **3.2.1.1 Isolates used for the test**
738 For this experiment we selected 10 *P. halstedii* isolates from the collection of MATE (former
739 SZIU) (isolates from 2014 and 2016) as well as we used some isolates from the 2017 collection
740 (Table 4).
741
742 **Table 4.** *Plasmopara halstedii* isolates used in the 10-isolate experiment during the fungicide
743 resistance tests

Isolate(code)	Locality (county)	Collection (year)	Pathotype (CVF)
I1	Tiszaföldvár	2017	704
I2	Mezőkovácsháza	2017	724
I3	Túrkeve	2017	700*
I4	Karácsond	2017	704*
I5	Bonyhád	2017	724*
I6	Pély	2017	704*
I7	Csongrád	2016	704
I8	Tiszafüred	2014	730
I9	Körösladány	2014	704
I10	Csanytelek	2014	730

744 *CVF (coded virulence formula) was determined during the pathotype identification of the thesis
745 (new results)

746 **3.2.1.2 Treatment of seeds with mefenoxam**

747 Seeds were treated with Apron XL 350 FS (350 g/L mefenoxam, Syngenta AG, Switzerland) as
748 per the European registered rate (3 mg/kg seeds) and evenly coated with the fungicide by mixing
749 in a beaker. Treated seeds were kept for drying at room temperature for three days. Non-treated
750 seeds were disinfected by immersion in a 1% Na-hypochlorite solution for 3-5 minutes and then
751 rinsed with running tap water.

752 **3.2.1.3 Preparation of inoculum and set of the 10-isolate experiment**

753 The preparation of inoculum as well as the method of inoculation was same as described in the
754 chapter "3.1.2, i.e., Propagation of inoculum using whole seedling immersion (WSI) method". For
755 non-inoculated plants, seedlings were incubated in a bidistilled water as a control. The seedlings
756 were sown in perlite in pots, containing 5 seedlings per pot.

757 The *P. halstedii* isolates of the I1–2, I3–6 and I7–10 codes were tested in separate experiments,
758 respectively, under the same conditions (Table 4). Each experiment was carried out twice with 10
759 replicates, respectively.

760 The following treatments and signs were used:

761 K0 – non-treated with mefenoxam, non-inoculated by *P. halstedii*; M – treated with mefenoxam,
762 non-inoculated by *P. halstedii*; I – non-treated with mefenoxam, inoculated by *P. halstedii*; MI –
763 treated with mefenoxam, inoculated by *P. halstedii*.

764 **3.2.1.4 Disease assessment and measuring plant heights**

765 The disease was evaluated once. Nine days after inoculation, plants were sprayed with bidistilled
766 water and covered with a dark polyethylene bag (Figure 4). Trays were placed in the dark for 24
767 h at 19 °C to induce sporulation. Plant heights were measured twice. The efficacy of mefenoxam

768 was calculated as the percentage of disease rate of treated and non-treated inoculated plants for
769 all isolates.

770 **3.2.1.5 Statistical analysis**

771 The data were subjected to ANOVA. Fisher's test at $P < 0.05$ was used for the mean separation.
772 The statistical analyses were performed using the software package Minitab (version 16.1.1.).
773

774 **3.2.2 Fungicide sensitivity test performed with 8 *P. halstedii* isolates by using soil drench**
775 **inoculation (SDI) method**

776 For this experiment we selected 8 *P. halstedii* isolates from the collection of MATE ((Table 5).
777 Treatment of seeds was the same as described in the chapters "3.1.2 and 3.2.1.2 i.e., treatment of
778 seeds with mefenoxam".

779 **Table 5.** *Plasmopara halstedii* isolates used in the 8-isolate experiment during the fungicide
780 resistance tests

Isolate code	Locality (county)	Year of collection	Pathotype (CVF)
1	Mezőkovácsháza (Békés)	2017	724
4	Kömlő (Heves)	2014	704
5	Doboz (Békés)	2014	704
6	Körösladány (Békés)	2014	714
7	Szeghalom (Békés)	2017	724
8	Pély (Heves)	2017	704*
9	Bonyhád (Tolna)	2017	724*
11	Rákóczifalva (Jász-Nagykun-Szolnok)	2012	704

781 *CVF (coded virulence formula) was determined during the pathotype identification of the thesis
782 (new results)
783

784 **3.2.2.1 Preparation of inoculum and inoculation using soil drench inoculation (SDI) method**

785 The propagation of inoculum was same as described in the chapter "3.1.2 i.e., Propagation of
786 inoculum and inoculation", except the concentration was adjusted to 50000 sporangia per mL
787 using a Burker counting chamber. Seedlings were sown in perlite in pots (d = 8 cm), containing 5
788 seeds per pot.

789 Three days after sowing, seedlings were inoculated by the soil drench method as described by
790 Trojanová et al. (2017) and Goossen and Sackston (1968) (Table 4). The sporangial suspension (2

791 mL per seedling) was pipetted directly onto the perlite surface of each pot containing the seedlings.
792 For the non-inoculated, bidistilled water was drenched over seedlings as a control.

793 **3.2.2.2 Set of the 8 isolate experiment and evaluation of disease**

794 The plants were kept at 16°C in the dark in a growth chamber for 24 h to ensure infection. After
795 inoculation, plants were grown in a growth chamber at 22°C with a 12 h photoperiod, light
796 irradiance of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Figure 3). The plants were watered regularly.
797 The *P. halstedii* isolates 1, 4, 5, 6, 7, 8, 9 and 11 were used during the experiment. Each experiment
798 was carried out twice with 10 replicates, respectively.

799 **3.2.2.3 Disease assessment**

800 Nine days after inoculation, plants were sprayed with bidistilled water and covered with a dark
801 polyethylene bag. Pots were placed in the dark for 24 h at 19°C to induce sporulation. The first
802 evaluation was based on white coating (sporangia) on cotyledons and pre-emergence damping-off,
803 referring to Disease 1. Twenty-one days after inoculation, a second evaluation was made according
804 to chlorosis along the veins of the true leaves and post-emergence damping-off, referring to
805 Disease 2. Plant heights were measured twice (Height 1 and 2) during each disease assessment.

806 **3.2.2.4 Microscopic observations**

807 Histological examinations of cross-sections of sunflower hypocotyls were performed using a
808 fluorescence microscope (Olympus, Japan; filter block BX 50, transmission > 515 nm). Twenty-
809 one days after inoculation, five sunflower hypocotyls were selected and fixed in FAA solution
810 (formalin-acetic acid-ethanol, 10:5:50 by volume) from each treatment. Thin cross-sections (15-
811 20 pieces) were cut with a razor blade from both upper and lower parts of the hypocotyl, and
812 examined for pathogen structures (hyphae, haustoria) and host tissue responses (hypersensitive
813 reaction, cell necrosis).
814 For the microscopic disease assessment, evaluation was conducted according to Bán et al. (2004)
815 i.e., a 0-4 scale was used for the appearance of pathogen structures and host tissue responses in
816 one, two, three, and four quarters of the cross-sections both in the cortical and pith parenchyma,
817 respectively.

818 **3.2.2.5 Statistical analysis**

819 Fisher's test at $P < 0.05$ was used for the mean separation. Differences in disease rates, host
820 characteristics (plant height) and host tissue responses (HR and cell necrosis) were assessed by
821 analyses of variance. (ANOVA) followed by the Tukey HSD (Honestly Significant Difference)
822 multiple comparison post-hoc test. Two-way ANOVA was used to examine the interaction
823 between treatment (non-treated, treated) and isolates. Using Ward's method hierarchical cluster
824 analysis was performed to group *P. halstedii* isolates based on their sensitivity to mefenoxam. To

825 examine the correlation between variables, Pearson's correlation coefficient was used for scale
826 variables (disease rates, heights) and Spearman's correlation coefficient was used for ordinal
827 variables (microscopic variables). The IBM SPSS Statistics 27 software was used to conduct the
828 statistical analysis.

829 **3.2.3 Assessing the effect of different concentrations of mefenoxam on 5 *P. halstedii* isolates**

830 The method of inoculation and fungicide treatment with different concentrations was the same as
831 described previously, in the chapters "3.1.2 and 3.2.1.2". The plants were grown in a growth
832 chamber with a photoperiod of 12 h at 22 °C, light irradiance of 100 $\mu E \cdot m^{-2} s^{-1}$.
833 *Plasmopara halstedii* isolates used in this experiment are listed in Table 6. Mád1, Kömlő,
834 Rákóczifalva and Csanytelek isolates were increased on untreated, while Mád2 isolate on
835 mefenoxam-treated sunflowers.

836 **Table 6.** Origin of *P. halstedii* isolates used in the experiment with different mefenoxam
837 concentration (pathotypes were identified previously as described in Bán et al. 2021)

Isolate code	Collection region (County)	Collection (year)	Pathotype
Mád1	Borsod-Abaúj-Zemplén	2014	700
Mád2	Borsod-Abaúj-Zemplén	2014	700
Kömlő	Heves	2014	704
Rákóczifalva	Jász-Nagykun-Szolnok	2012	704
Csanytelek	Csongrád-Csanád	2014	730

838

839 The whole seedling immersion (WSI) method by Cohen and Sackston (1973) was used for this
840 experiment. The *P. halstedii* isolates were tested in two subsequent experiments with two
841 replicates for each test.
842 The following treatments were used in the experiment:

843 - Zero control: Seedlings treated with bidistilled water.
844 - Infected control: Seedlings inoculated with *P. halstedii* sporangial suspension.
845 - Mefenoxam (1 mg/kg) treated seeds treated with bidistilled water.
846 - Mefenoxam (1 mg/kg) treated seeds inoculated with *P. halstedii* sporangial suspension.
847 - Mefenoxam (3 mg/kg) treated seeds treated with bidistilled water.
848 - Mefenoxam (3 mg/kg) treated seeds inoculated with *P. halstedii* sporangial suspension.

849 • Mefenoxam (9 mg/kg) treated seeds treated with bidistilled water.
850 • Mefenoxam (9 mg/kg) treated seeds inoculated with *P. halstedii* sporangial suspension.
851 • Mefenoxam (18 mg/kg) treated seeds treated with bidistilled water.
852 • Mefenoxam (18 mg/kg) treated seeds inoculated with *P. halstedii* sporangial suspension.
853 • Mefenoxam (30 mg/kg) treated seeds treated with bidistilled water.
854 • Mefenoxam (30 mg/kg) treated seeds inoculated with *P. halstedii* sporangial suspension.
855 The evaluation of the disease is the same as described in the chapter, "3.2.2.3".
856 ANOVA followed by a post-hoc Tukey test was performed to compare the different treatments in
857 R software v 3.4.0 R Core Team, while graphs were made in Excel.
858

859 **3.3 Efficacy of neem-derived pesticides to restrict sunflower downy mildew**

860 **3.3.1 *In-vitro* experiment: Examination of the effect of neem-derived pesticides on *P. halstedii***
861 **sporangial germination**

862 Sunflower leaves infected by two *P. halstedii* isolates (Mád and Rákóczifalva) stored in deep
863 freezer were soaked in 20 mL bidistilled water to release the sporangia. One milliliter (mL) of
864 sporangia suspension was mixed with 0.5 mL of each tested concentrations of neem leaf extract
865 or azadirachtin solutions, or with 0.5 mL of mefenoxam in an Eppendorf tube. It was agitated
866 gently to mix uniformly and avoid bursting of sporangia, and was incubated at 16 °C for 24 h in
867 the dark in a thermostat. After a 24 h incubation period, samples were observed with a microscope
868 at 200× magnification, to check the effect of neem derived pesticides on the sporangia morphology
869 and release of zoosporangia. Microscopic examination was done for each tested treatment by
870 counting first 50 sporangia/treatment. The experiment was replicated five times with each
871 treatment. Microscopic examination of sporangia in bidistilled water (BW) served as a negative
872 control.
873 The following treatments were used for *in-vitro* experiment:
874 • Control – bidistilled water + *P. halstedii* sporangial suspension
875 • 3 mg/kg mefenoxam + *P. halstedii* sporangial suspension
876 • 10% Neem leaf extract solution + *P. halstedii* sporangial suspension
877 • 20% Neem leaf extract solution + *P. halstedii* sporangial suspension
878 • 0.01% NeemAzal solution + *P. halstedii* sporangial suspension
879 • 0.1% NeemAzal solution + *P. halstedii* sporangial suspension
880

881 **3.3.2 *In-vivo* experiment: Effect of neem-derived pesticides on *P. halstedii* isolates (Mád and**
882 **Rákóczifalva) in sunflower**

883 **3.3.2.1 Preparation of neem leaf extract (NLE)**

884 The methodology for preparing neem leaf extract was followed according to Doshi et al. (2018)
885 with slight modifications. The air-dried neem leaves were ground into powder using an electric
886 blender. Two concentrations of 10% and 20% (w/v) were prepared by soaking 10 g and 20 g of
887 neem leaf powder, respectively, in 100 mL of distilled water overnight, and then followed by
888 filtration through a non-sterile cheesecloth to remove the coarse leaf materials. The filtered extract
889 was centrifuged at 5000 rpm for 5 min to remove the remaining particles and obtain a clear extract.

890 **3.3.2.2 Preparation of azadirachtin (NeemAzal T/S) (AZA)**

891 A working concentration of 0.01% and 0.1% were prepared of NeemAzal T/S obtained from
892 Trifolio Gmbh, Germany, containing (1% azadirachtin), a registered plant protection commercial
893 product in the European Union, by dissolving 1 mL and 10 mL NeemAzal T/S in 100 mL of
894 distilled water, respectively (Doshi et al. 2020).

895 **3.3.2.3 Germination process and treatments**

896 The sterilization method was the same as discussed previously. After sterilization, seeds were pre-
897 soaked in different concentrations of Neem leaf extract (10 and 20%) and Neem Azal (0.01 and
898 0.1%) for 4 hours. After 4 hours, seeds were placed on wet filter paper, and germinated at 20 °C
899 for 2 to 3 days (Doshi et al. 2020).

900 The whole seedling immersion (WSI) method by Cohen and Sackston (1973) was used for the
901 experiment, as described previously. The *P. halstedii* isolates were tested in two subsequent
902 experiments with two replicates for each test.

903 The following treatments were used in the experiment:

904 - Non-treated seedlings inoculated with *P. halstedii* sporangial suspension.
905 - Non-treated seedlings treated with bidistilled water (BW).
906 - Treated seeds with mefenoxam (3 mg/kg) inoculated with *P. halstedii* sporangial
907 suspension.
908 - Treated seeds with mefenoxam (3 mg/kg) treated with bidistilled water (BW).
909 - Seedlings pre-treated with AZA 0.01% and inoculated with *P. halstedii* sporangial
910 suspension.
911 - Seedlings pre-treated with AZA 0.01% and treated with bidistilled water (BW).
912 - Seedlings pre-treated with AZA 0.1% and inoculated with *P. halstedii* sporangial
913 suspension.
914 - Seedlings pre-treated with AZA 0.1% and treated with bidistilled water (BW).

- Seedlings pre-treated with NLE 10% and inoculated with *P. halstedii* sporangial suspension.
- Seedlings pre-treated with NLE 10% and treated with bidistilled water (BW).
- Seeds pre-treated with NLE 20% and inoculated with *P. halstedii* sporangial suspension.
- Seedlings pre-treated with NLE 20% and treated with bidistilled water (BW).

For neem-derived pesticide experiment, thin cross-sections of both upper and lower parts of the hypocotyls were made. The followed was the same as discussed in chapter '3.2.2.4'.
The disease was evaluated twice. The evaluation of the disease is the same as described in the chapter "3.2.2.3".
For both, *in vitro* and *in vivo* experiments, ANOVA followed by a post-hoc Tukey test was performed to compare the different treatments in R software v 3.4.0 R Core Team.

942 # 4. RESULTS

943 ## 4.1 Pathotype identification of *P. halstedii* isolates collected in Hungary (2017-2019)

944 The results of the sunflower downy mildew isolates collected in Hungary between 2017 and 2019
945 for their pathotype are shown in Table 7.

946 Out of the 22 *P. halstedii* isolates, nine isolates were characterized as pathotype 704, four as
947 pathotype 700, three as pathotype 724, one as pathotype 714, one as pathotype 730 and four as
948 pathotype 734 (Table 7). Among all pathotypes, pathotype 704 was the most widespread in
949 collected samples. Differential lines Iregi szürke csíkos, RHA-265, and RHA-274 were completely
950 infected by *P. halstedii* isolates for all samples. Most of these infected plants showed damping-off
951 by the time of the second evaluation, so these lines were highly susceptible to the examined
952 pathotype of sunflower downy mildew. During the study, 734 was identified as a new pathotype
953 in Hungary (Nisha et al. 2021, see details below).

954 **Table 7.** Virulence character of *P. halstedii* isolates collected from Hungary in 2017-2019.

Isolate number	Sunflower genotype (*Pl* gene)	Locality	CVF of isolate (pathotype)
1	*Pl*6	Karácsond (HU)	704
2	unknown	Martfű (HU)	704
3	*Pl*6	Galgahévíz (HU)	704
4	*Pl*6	Csongrád (HU)	704
5	volunteer	Hatvan (HU)	700
6	volunteer	Hatvan (HU)	704
7	unknown	Pély (HU)	704
8	*Pl*6	Túrkeve (HU)	700
9	*Pl*6	Bonyhád (HU)	724
10	*Pl*6	Bonyhád (HU)	704
11	*Pl*6	Vésztő (HU)	724
12	Experimental line	Szeged (HU)	714
13	*Pl*6	Abony (HU)	704
14	*Pl*6	Tápé (HU)	704
15	*Pl*6	Szamoskér (HU)	700
16	*Pl*6	unknown (HU)	700
17	*Pl*6	Békésszentandrás (HU)	724
18	*Pl*6	Léh (HU)	**734***

19	*Pl*6	Bucsa (HU)	**734***
20	*Pl*6	Kertészsziget (HU)	**734***
21	*Pl*6	Kötegyán (HU)	730
22	*Pl*6	Vanyarc (HU)	**734***

955 * 734 pathotype has been reported newly in Hungary and published in Nisha et al. (2021)

956 **4.1.1 Identification of a new pathotype, 734, in Hungary**

957 All four isolates examined caused disease on differential lines HA-304, RHA265, RHA-274, PMI-
958 3, PM-17, and HA-335, whereas the other lines showed no symptoms and signs of sunflower
959 downy mildew. Summing the scores given according to the reactions of the differential lines by
960 each triplet, the examined *P. halstedii* isolates were identified as pathotype 734 (Table 8). This
961 pathotype is likely widespread in Hungary because it was detected from three different regions.

962 **Table 8.** Pathotype characterization of *P. halstedii* isolate 734 (S = Susceptible, R = Resistant).

Differential lines	**Reaction of plants**	**Score**	**Pathotype Code (CVF)**
Iregi szürke csíkos	S	1	**7**
RHA-265	S	2	
RHA-274	S	4	
PMI-3	S	1	**3**
PM-17	S	2	
803-1	R	0	
HAR-4	R	0	**4**
QHP-2	R	0	
HA-335	S	4	

963

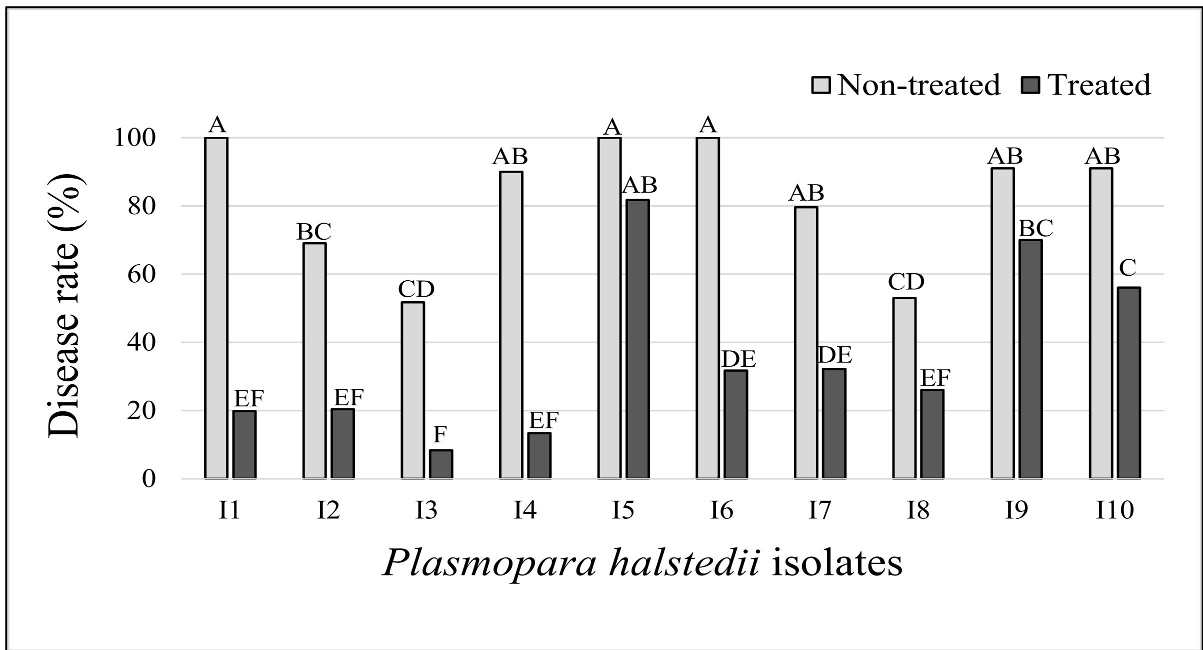
4.2 Fungicide sensitivity tests

4.2.1 Fungicide sensitivity tests performed on 10 *P. halstedii* isolates by using the whole seedling immersion (WSI) method

The disease rates of the different *P. halstedii* isolates on the mefenoxam-treated and non-treated sunflowers are shown in Figure 5. Seven out of the ten isolates caused relatively high disease rates (ranging from 20 to 80%) on the mefenoxam-treated and inoculated sunflower plants with *P. halstedii*. Among these, the highest infection rates were found with the I5 (pathotype 724 from Bonyhád), I9 (pathotype 704 from Körösladány) and I10 (pathotype 730 from Csanytelek) isolates. The downy mildew isolates showing sensitivity to mefenoxam were I1 (pathotype 704 from Tiszaföldvár), I3 (pathotype 700 from Túrkeve) and I4 (pathotype 704 from Karácsond). All the non-treated and inoculated plants with isolates I1, I5 and I6 showed a damping-off by the end of the experiment. The efficacy (%) of mefenoxam on the different *P. halstedii* isolates was calculated as the percentage reduction in the disease rate relative to the non-treated infected control. Mefenoxam performed poorly (18–40%) on three *P. halstedii* isolates (I5, I9, I10) and gave moderate (41–60%) protection against two isolates (I8, I7). The protection was good (61–80%) to excellent (> 81%) on five isolates (I1, I2, I3, I4, I6).

As the stunting of the infected plant is a significant symptom of *P. halstedii*; hence, the plant height was measured twice for some isolates during the experiments (Figures 6 and 7). There was no significant difference between the heights of the non-inoculated, mefenoxam treated and non-inoculated, non-treated plants in any of the experiments at any time of recording the information. Furthermore, the mefenoxam-treated and inoculated sunflowers grew similarly to the non-inoculated ones at the first evaluation (Figure 6). The plant heights were significantly lower for the non-treated sunflowers inoculated with the *P. halstedii* isolates, I1, I4, I5 and I6, than that of treated plants at the first evaluation. The non-treated plants inoculated with I1, I5 and I6 isolates showed a damping-off by the time of the second evaluation (Figure 7). The non-treated, inoculated plants with the I2 and I3 isolates showed significantly lower heights than the treated ones at the second evaluation.

991

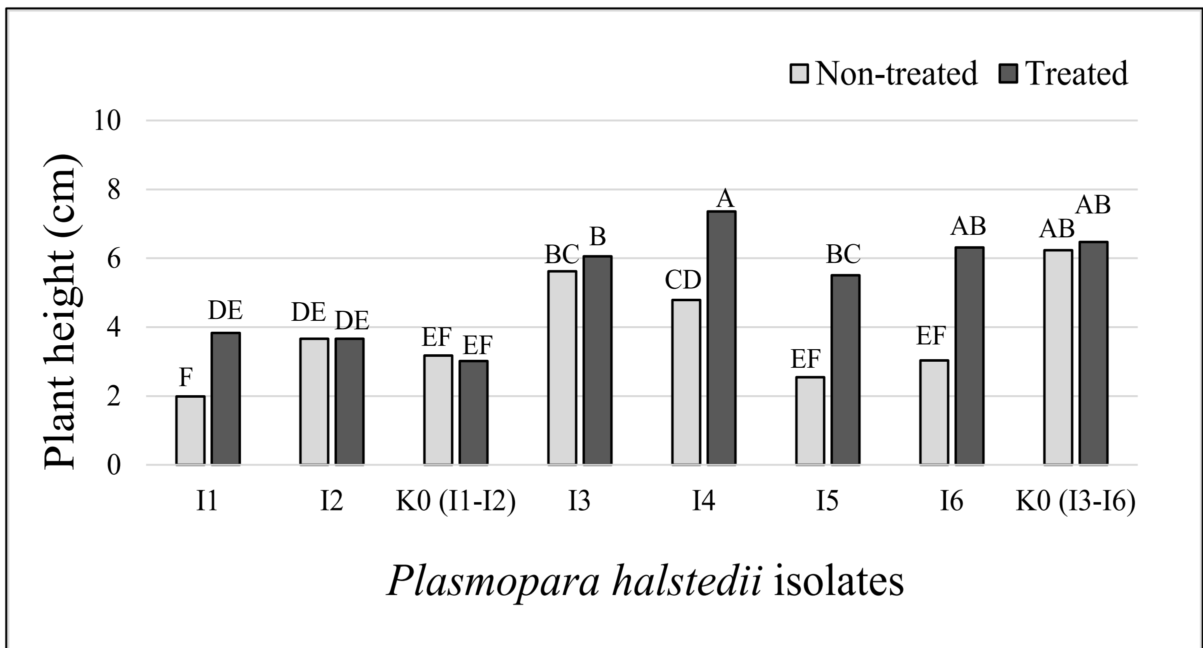


992 **Figure 5.** Disease rates (%) on the sunflowers (treated and non-treated with mefenoxam)
993 inoculated by the different isolates of *Plasmopara halstedii* 9 days after inoculation.

994 Non-treated – non-treated with mefenoxam and inoculated with *P. halstedii*; treated – treated with
995 mefenoxam (3 mg/kg seed) and inoculated with *P. halstedii*; ANOVA was performed with Fisher's test; the
996 bars sharing the same letter are not significantly different. Isolate codes are in Table 4.

997

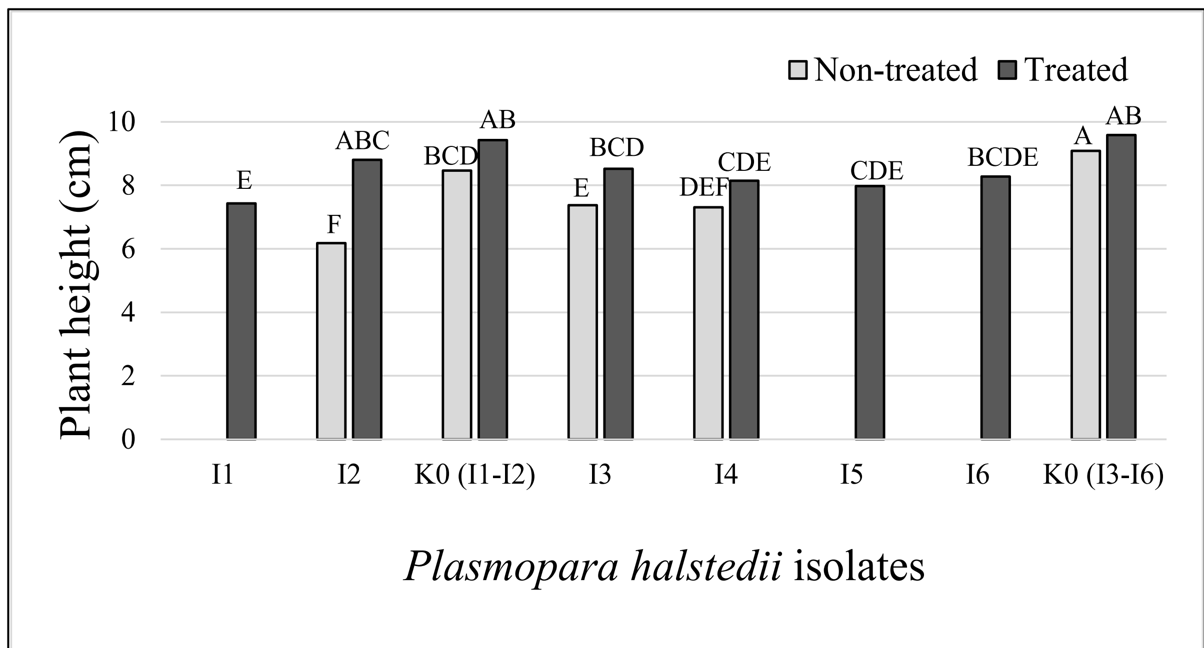
998



999 **Figure 6.** Plant heights of the mefenoxam-treated and non-treated sunflowers 9 days after
1000 inoculation.

1001 Non-treated – non-treated with mefenoxam and inoculated with *P. halstedii*; treated – treated with
1002 mefenoxam (3 mg/kg seed) and inoculated with *P. halstedii*; K0 (I1 – I2) and K0 (I3 – I6) – non-treated
1003 and non-inoculated with *P. halstedii* for I1–I2 and I3–I6, respectively; ANOVA was performed with
1004 Fisher's test; the bars sharing the same letter are not significantly different. Isolate codes are in Table 4.

1005



1006 **Figure 7**. Plant heights of the mefenoxam-treated and non-treated sunflowers 21 days after
1007 inoculation with *P. halstedii.*

1008 Non-treated – non-treated with mefenoxam and inoculated with *P. halstedii*; treated – treated with
1009 mefenoxam (3 mg/kg seed) and inoculated with *P. halstedii*; K0 (I1 – I2) and K0 (I3 – I6) – non-treated
1010 and non-inoculated with *P. halstedii* for I1–I2 and I3–I6, respectively; ANOVA was performed with
1011 Fisher's test; the bars sharing the same letter are not significantly different. Isolate codes are in Table 4.

1012

1013 **4.2.2 Fungicide sensitivity tests performed on 8 *P. halstedii* isolates by using the soil drench**
1014 **inoculation method**

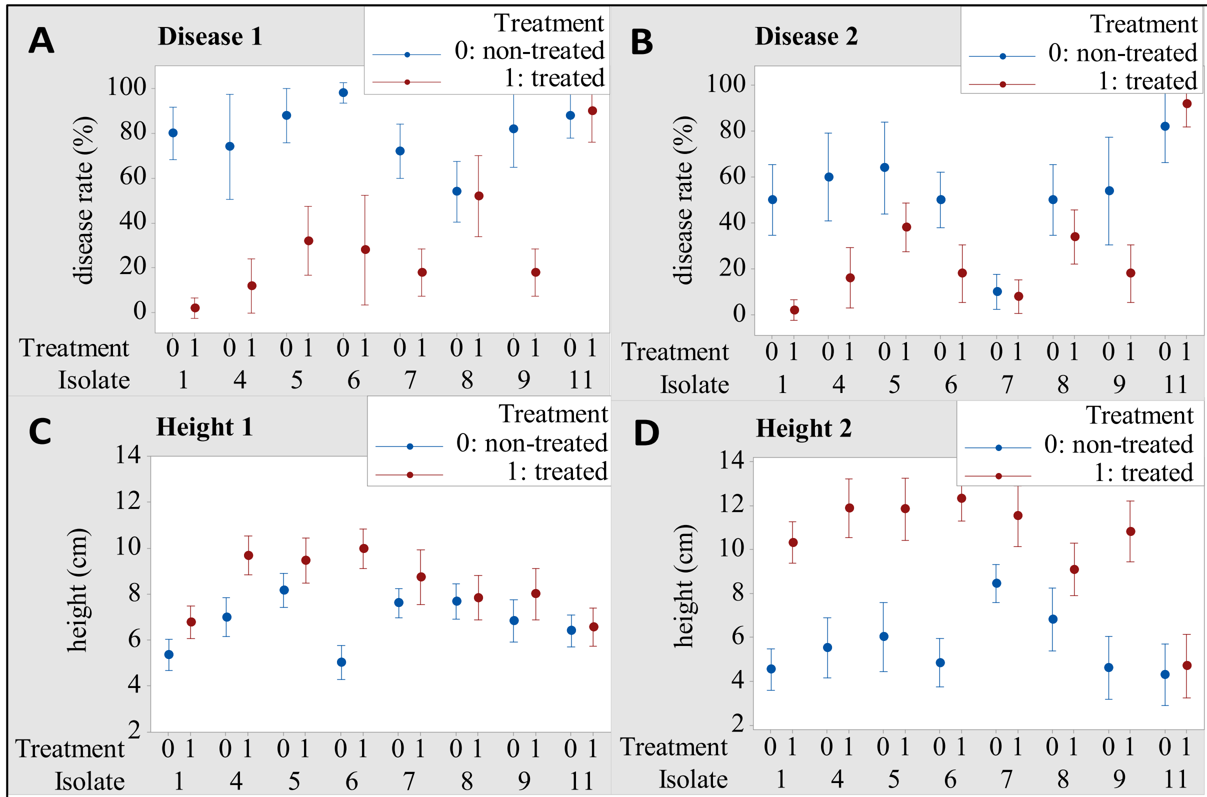
1015 **4.2.2.1 Disease rates and plant heights**

1016 Disease rates (%) and heights of mefenoxam treated and non-treated sunflower plants inoculated
1017 with different *Plasmopara halstedii* isolates are shown in Figure 8. According to the sporulation
1018 of the pathogen on the cotyledons and pre-emergence damped-off plants (Disease 1, Figure 8A),
1019 mefenoxam-treated sunflowers inoculated with the isolates 1, 4, 5, 6, 7, and 9 showed significantly
1020 lower infection rates compared to non-treated ones. However, there were no significant differences
1021 in disease rates between treated and non-treated plants inoculated with isolates 8 and 11. The
1022 situation was similar with Disease 2 (ratio of chlorotic, post-emergence damped-off plants and
1023 healthy sunflowers, Figure 8B), but there was no difference in the disease rate of treated and non-
1024 treated plants inoculated with isolates 7 in addition to isolates 8 and 11.

1025 Plants of mefenoxam treated sunflowers inoculated with *P. halstedii* isolates 1, 4, 5, 6 were
1026 significantly higher than that of the non-treated inoculated plants nine days after inoculation
1027 (Figure 8C). On the contrary, there was no significant difference in plant heights between treated
1028 and non-treated sunflowers inoculated by isolates 7, 8, 9, and 11. However, by the second

1029 recording date, the height of the treated plants was significantly higher than the non-treated plants
1030 for all isolates except 11 (Figure 8D).

1031 For all parameters tested (Disease 1-2, Height 1-2), the interaction between isolate and treatment
1032 was significant (for Disease 1: $F=12.06$, $p<0.001$, for Disease 2: $F=5.36$, $p<0.001$, for Height 1:
1033 $F=6.61$, $p<0.001$, for Height 2: $F=7.37$, $p<0.001$), i.e., the impact of treatment varied between
1034 isolates.



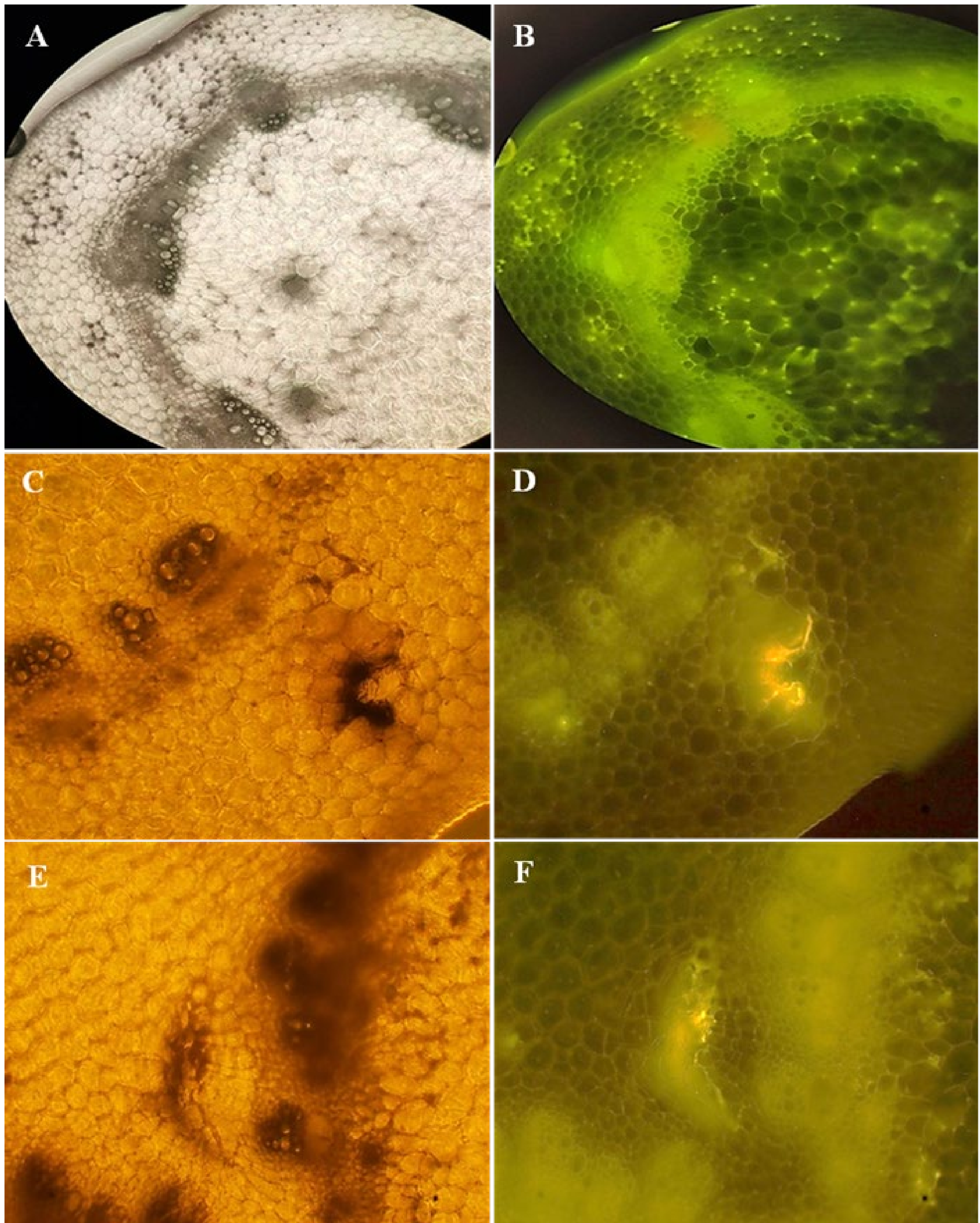
1035 **Figure 8.** Disease rates (A, B) and heights (C, D) of mefenoxam treated and non-treated
1036 sunflower plants inoculated with different *Plasmopara halstedii* isolates.
1037
1038 Disease 1: ratio of sporulating, pre-emergence damped-off plants and healthy sunflowers nine days after
1039 inoculation.
1040 Disease 2: ratio of chlorotic, post-emergence damped-off plants and healthy sunflowers 21 days after
1041 inoculation.
1042 Height 1: height of sunflowers nine days after inoculation (heights of damped-off plants were taken as
1043 zero).
1044 Height 2: height of sunflowers 21 days after inoculation (heights of damped-off plants were taken as zero).
1045 Treatment: non-treated (0) and treated (1) with mefenoxam (3 mg/kg seed).
1046 Isolate: code of *Plasmopara halstedii* isolates used in the experiment (1, 4, 5, 6, 7, 8, 9, 11) (for more
1047 details, see Table 5)
1048 Bars represent the 95 % confidence intervals ($p \geq 0,5$). Individual standard deviations were used to calculate
1049 the intervals.
1050

1051 ### 4.2.2.2 Microscopic observations of host tissue responses

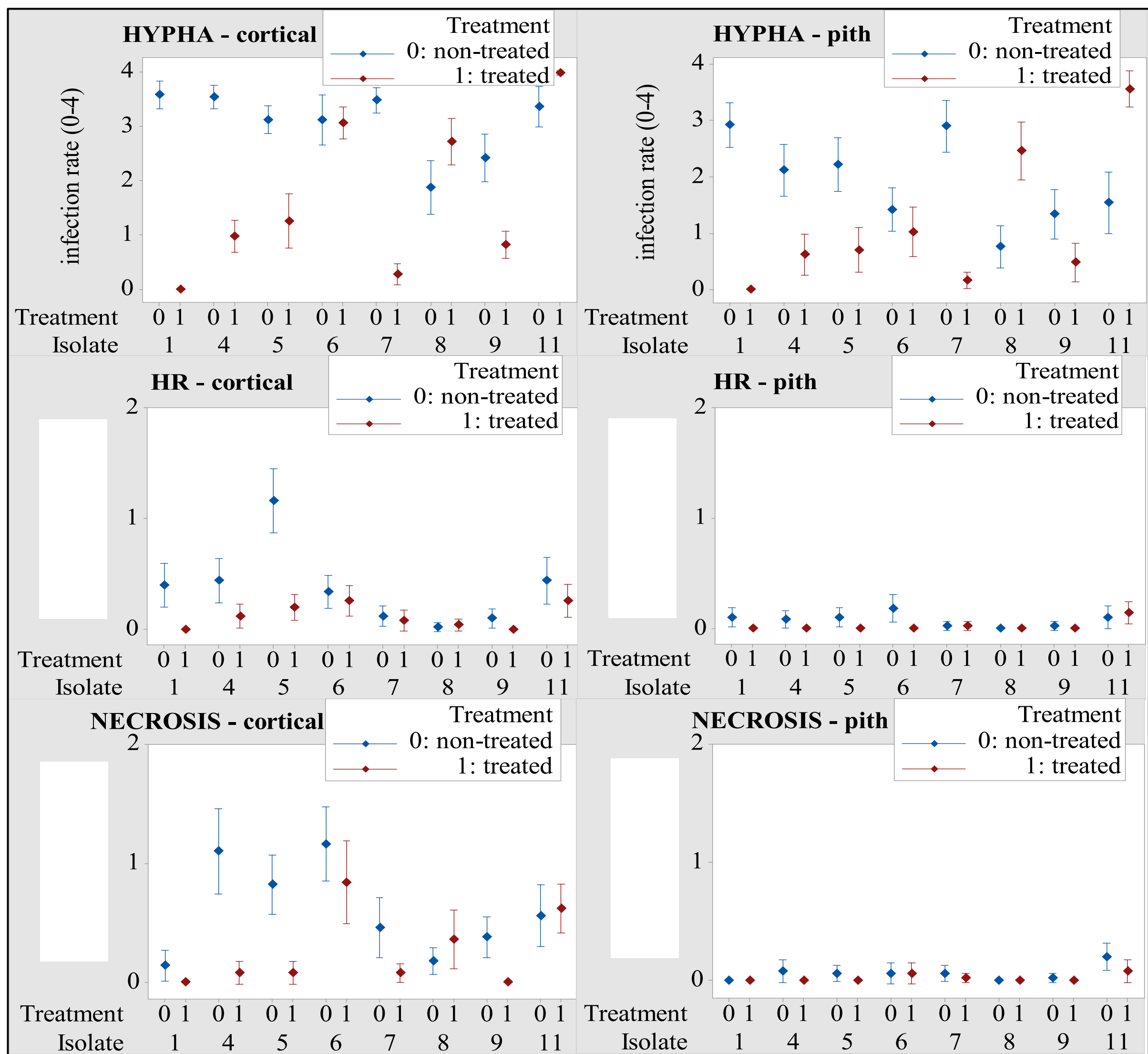
1052 Host tissue responses of sunflowers to infection by *P. halstedii* in hypocotyl cross-sections are
1053 shown in Figure 9. Similar tissue responses were observed in most treated and non-treated plants
1054 infected with different isolates, but the intensity of the pathogenic spread and plant responses were

1055 variable. In general, intercellular hyphae and intracellular haustoria were detected in the hypocotyl
1056 of non-treated plants both in the cortical and the pith parenchyma 21 days after inoculation (Figure
1057 9A). Under UV light, fluorescence appeared in the intercellular spaces around hyphae, giving the
1058 image a dotted look (Figure 9B). By contrast, cell browning under normal light (Figure 9C) and
1059 an intense fluorescence of cells showing a hypersensitive-like reaction (Fig 9D) could be detected
1060 in cross-sections of several mefenoxam-treated sunflowers. Moreover, the development of cellular
1061 necrosis by vigorous cell division (Figure 9E) and the strong fluorescent response of surrounding
1062 cells (Figure 9F) was also frequently observed in treated and inoculated plants.
1063 The rate of pathogen hyphal spread and host tissue responses are shown in Figure 10. Hyphae were
1064 able to spread to a significantly greater extent in the cortical and pith parenchyma of non-treated
1065 plants inoculated with isolates 1, 4, 5, and 7 compared to mefenoxam-treated plants (Figure 10A
1066 and B). In contrast, more hyphae were found in the cortical and pith parts of mefenoxam-treated
1067 sunflowers inoculated with *P. halstedii* isolate 8 than in non-treated ones. The situation was similar
1068 for the appearance of hyphae of isolate 11 in the pith. In addition, hyphae were significantly more
1069 abundant in the cortical part of non-treated than treated sunflowers inoculated with isolate 9,
1070 whereas there was no significant difference in hyphal distribution between treated and non-treated
1071 sunflowers for isolate 6 (Figure 10A and B).
1072 Generally, fluorescence microscopy of cross-sections of sunflower hypocotyls revealed a
1073 relatively higher rate of hypersensitive-like reaction and necrosis (cell death) in the cortical than
1074 in the pith parenchyma in this experiment (Figure 10C-F). The hypersensitive reaction was
1075 prominent in non-treated plants inoculated with isolate 5 and to a smaller extent in non-treated
1076 sunflowers inoculated with isolates 1, 4, 6, and 11 in the cortical parenchyma (Figure 7C and D).
1077 However, it was not significant for the two latter compared to mefenoxam-treated plants. The
1078 occurrence of cell necrosis in the cortical part was intensive in non-treated plants inoculated with
1079 isolates 4, 5, and 6. For the latter, it was not significant compared to mefenoxam-treated sunflowers
1080 (Figure 10E). Necrosis in the pith parenchyma cells was minimal in each sample (Figure 10F).

1081



1082 **Figure 9**. Light micrographs of mefenoxam-activated resistance responses in hypocotyl cross-
1083 sections of sunflower. Hyphae of *Plasmopara halstedii* invade cells of non-treated, inoculated
1084 susceptible plants (cv. Iregi szürke csíkos) without any host tissue responses in normal (A) and in
1085 UV light (B) (λ = 485 nm), at 21 dpi. Browning (C), autofluorescence (hypersensitive reaction)
1086 (D), and necrosis (E: normal light, F: UV light) of cortical parenchyma cells neighboring invaded
1087 cells as a host tissue response to the pathogenic attack of mefenoxam-treated, inoculated plants, at
1088 21 dpi. Scale bar = 100 µm

1089


1090 **Figure 10.** Occurrence of the pathogen hypha (A, B) and host tissue responses such as
1091 hypersensitive reaction (C, D) and necrosis (E, F) in the cortical and pith parenchyma of
1092 mefenoxam treated and non-treated sunflower plants inoculated with *Plasmopara halstedii*.

1093 Treatment: non-treated (0) and treated (1) with mefenoxam (3 mg/kg seed).
1094 Isolate: code of *Plasmopara halstedii* isolates used in the experiment (1, 4, 5, 6, 7, 8, 9, 11)
1095 The infection rate and the intensity of the host reaction were measured on a 0-4 scale.
1096 Vertical lines represent 95% confidence intervals (95% CI) of the mean values of disease rates and heights
1097

1098 **4.2.2.3 Assessing the sensitivity of *Plasmopara halstedii* isolates to mefenoxam**

1099 Cluster analyses of sunflowers based on disease rates and plant heights inoculated by different *P.*
1100 *halstedii* isolates are shown in Table 9. Four distinct clusters could be identified determined by
1101 macroscopic parameters. Cluster 1 includes non-treated plant samples inoculated with isolates 5,
1102 6, 9, 11, and mefenoxam-treated plants from 11, which were found to have high infection levels
1103 in both sampling periods. Therefore, the pathogen was able to penetrate the upper parts of these
1104 sunflowers. Plant heights were the lowest in this group. In Cluster 2 are samples of the other part
1105 of non-treated and inoculated plants, where the first infection value (Disease 1) was relatively high,
1106 like Cluster 1. However, unlike the first cluster, the second time point for disease assessment

1107 (Disease 2) resulted in much lower infection values and less plant dwarfing in Cluster 2 members
1108 (Table 9). In this case, the pathogen could only penetrate to a lesser extent above the hypocotyl.
1109 Clusters 3 and 4 mainly include samples of inoculated plants treated with mefenoxam. In contrast
1110 to the initial infection rates, there was no significant difference between the two clusters in the
1111 second survey. However, the plant height values were significantly higher for Cluster 3 members
1112 (Table 9).

1113 **Table 9.** Cluster analyses of sunflowers based on disease rates and plant heights inoculated by
1114 different *P. halstedii* isolates.

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Disease 1 (%)	90.2 ± 6.9 **d**	72.2 ± 12 **c**	20.4 ± 12.3 **a**	38.2 ± 13.3 **b**
Disease 2 (%)	74.5 ± 10.8 **C**	29.6 ± 10.4 **B**	15.9 ± 8.6 **A**	27.3 ± 10.2 **AB**
Height 1 (cm)	6.0 ± 0.8 **a**	7.1 ± 0.4 **b**	9.7 ± 0.8 **c**	7.4 ± 0.6 **b**
Height 2 (cm)	4.0 ± 1.0 **A**	7.5 ± 0.6 **B**	11.7 ± 1.1 **D**	9.3 ± 0.9 **C**

1115
1116 Data represent the means of variables for each cluster. Values followed by means represent standard deviation.
1117 Different letters (e.g., A, a) indicate significant differences based on the Tukey HSD post-hoc test ($p<0.05$).
1118 Cluster 1: isolates 1, **5, 6, 9, 11** non-treated, **11** treated.
1119 Cluster 2: isolates **1**, **4**, **7**, **8** non-treated.
1120 Cluster 3: isolates **1, 4, 5, 6**, 7, 9 treated.
1121 Cluster 4: isolates 1, 4, 5, 6, **7, 8, 9** treated, 8, 9 non-treated.
1122 Bold isolate numbers indicate dominance of that isolate in that cluster compared to other clusters.
1123

1124 Cluster analyses of sunflowers based on the examined microscopic variables inoculated by
1125 different *P. halstedii* isolates is presented in Table 10. Three distinct clusters could be identified
1126 by microscopic parameters, such as hyphal spread, the occurrence of hypersensitive-like reaction,
1127 and the development of necrosis in the cortical and pith parenchyma. Samples of non-treated
1128 inoculated plants are in the first two clusters, while mefenoxam-treated plants can be found in all
1129 three clusters. Moreover, treated plants inoculated with isolates 4 and 5 are equally represented in
1130 the first two clusters.

1131 For Cluster 1 samples, the pathogen could invade both the cortical and pith parenchyma (Table
1132 10). Not only the spread of hyphae but also the HR and necrosis in different tissue sections were
1133 significant in Cluster 1 samples compared to the other two clusters. Besides non-treated ones,
1134 treated sunflowers inoculated with the *P. halstedii* isolates 8 and 11 are included in the first cluster.
1135 Unlike the sunflowers in the first cluster, the distribution of hypha of samples in Cluster 2 was
1136 accompanied by HR and necrosis only in the cortical parenchyma but not in the pith. Most of the
1137 treated sunflower samples, except for isolates 6, 8, and 11, are in Cluster 3, with little hyphae
1138 detected in the cortical tissues. No plant response was detected in these sunflowers.
1139

1140 **Table 10.** Cluster analyses of sunflowers based on the examined microscopic variables inoculated
1141 by different *P. halstedii* isolates.
1142

Variables	Cluster 1	Cluster 2	Cluster 3
H_Cort	3.7 ± 0.3 **C**	3.0 ± 0.5 **B**	0.2 ± 0.2 **A**
HR_Cort	0.4 ± 0.4 **c**	0.2 ± 0.2 **b**	0 **a**
NEC_Cort	0.7 ± 0.5 **C**	0.5 ± 0.4 **B**	0 **A**
H_Pith	3.6 ± 0.4 **c**	0.5 ± 0.3 **b**	0 **a**
HR_Pith	0.1 ± 0.2 **B**	0 **A**	0 **A**
NEC_Pith	0.1 ± 0.2 **b**	0 **a**	0 **a**

1143
1144 Data represent the means of variables for each cluster. Values followed by means represent standard deviation.
1145 Different letters (e.g., A, a) indicate significant differences based on the Tukey HSD post-hoc test (p<0.05).
1146 Cluster 1: isolates **1, 4, 5, 7** non-treated, **8, 11** treated
1147 Cluster 2: isolates **4, 5, 6, 8, 9, 11** non-treated, **6** treated
1148 Cluster 3: isolates **1, 4, 5, 7, 9** treated.
1149 Bold isolate numbers indicate dominance of that isolate in that cluster compared to other clusters. The underlined
1150 isolates were equally represented in the clusters concerned.
1151

1152 **4.2.2.4 Correlations among macroscopic and microscopic parameters**

1153 The results of Pearson correlation based on the examined macroscopic variables (disease rates,
1154 plant heights) are shown in Table 11. During the second evaluation, a strong negative correlation
1155 was found between the disease rate and plant height values of both non-treated and treated plants.
1156 Similarly, there was a strong negative correlation between the initial disease rates and the final
1157 plant height values of treated plants in the experiment. In contrast, a high positive correlation could
1158 be detected between the initial and final plant height data of both treated and non-treated plants.
1159 In addition, a strong positive correlation was found between the initial and final disease values of
1160 mefenoxam-treated sunflowers.
1161

1162 **Table 11.** Pearson correlation among the examined variables (disease rates, plant heights).

Variable	Disease 1	Disease 2	Height 1	Height 2
Panel A: Non-treated (n=80)				
Disease 1	1	0..346**	-0.465**	-0.550**
Disease 2		1	-0.439**	**-0.713****
Height 1			1	**0.737****
Height 2				1

Panel B: Treated (n=80)				
Disease 1	1	**0.701****	-0.368**	**-0.700****
Disease 2		1	-0.329**	**-0.722****
Height 1			1	**0.741****
Height 2				1

1163
1164 Disease 1: ratio of sporulating, damped-off plants and healthy sunflowers nine days after inoculation.
1165 Disease 2: ratio of chlorotic, damped-off plants and healthy sunflowers 21 days after inoculation.
1166 Height 1: height of sunflowers nine days after inoculation (heights of damped-off plants were taken as zero).
1167 Height 2: height of sunflowers 21 days after inoculation (heights of damped-off plants were taken as zero).
1168 Treatment: non-treated and treated with mefenoxam (3 mg/kg seed).
1169 **Correlation is significant at the 0.01 level (2-tailed).
1170 Values in bold indicate a strong correlation between variables.
1171

1172 The Spearman correlation of the examined microscopic variables is presented in Table 12. There
1173 was a strong positive correlation in the occurrence of hyphae in different parenchymatic plant parts
1174 (cortical and pith) of both non-treated and treated inoculated sunflowers. Moreover, strong positive
1175 correlations were found among the presence of hyphae in the cortical parenchyma tissues and the
1176 appearance of hypersensitive reaction and necrosis, respectively, in treated plants. In addition, a
1177 strong positive correlation could be confirmed for the establishment of necrosis in the cortical part
1178 and the occurrence of hyphae in the pith of mefenoxam-treated and inoculated sunflowers.
1179

1180 **Table 12.** Spearman correlation among the examined microscopic variables
1181

Variable	H_Cort	HR_Cort	NEC_Cort	H_Pith	HR_Pith	NEC_Pith
Panel A: Non-treated (n=200)						
H_Cort	1	0,211**	0,291**	**0,508****	0,158**	0,150**
HR_Cort		1	0,240**	0,193**	**0,375****	0,080
Nec_Cort			1	0,223**	0,155**	0,172**
H_Pith				1	0,156**	0,248**
HR_Pith					1	0,106*
Nec_Pith						1
Panel B: Treated (n=200)						
H_Cort	1	**0,327****	**0,488****	**0,759****	0,174**	0,153**
HR_Cort		1	0,072	0,213**	0,241**	0,029
Nec_Cort			1	**0,547****	0,079	0,180**
H_Pith				1	0,204**	0,169**

HR_Pith					1	0,129*
Nec_Pith						1

H: hyphae of *Plasmopara halstedii*
HR: hypersensitive reaction of invaded cells
Nec: necrosis
Cort: cortical parenchyma
Pith: pith parenchyma
**Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Values in bold indicate a strong correlation between variables.

4.2.3 Effect of different concentrations of mefenoxam on 5 *P. halstedii* isolates in sunflower

4.2.3.1 Disease rates

Disease 1 and 2 values are shown in Figures 11 and 12. The values of non-treated, inoculated plants with Mád1 and Mád2 isolates were significantly higher than those of other *P. halstedii* isolates. Disease values gradually decreased for all plants inoculated with different isolates by increasing mefenoxam concentration. Disease values of plants inoculated with Kömlő, Rákóczifalva, and Csanytelek isolates were halved already at 3 mg/kg mefenoxam concentration compared to control plants. In contrast, in the Mád isolates, the halving occurred at 18 mg/kg concentration.

Interestingly, in sunflowers inoculated with Mád isolates, there was no difference in Disease 1 and 2 values between 18 and 30 mg/kg treatments with mefenoxam. In addition, no or minimal infection was found on plants inoculated with Kömlő, Rákóczifalva, and Csanytelek isolates at 9, 18, and 30 mg/kg mefenoxam concentrations during the assessment period (Figures 11 and 12).

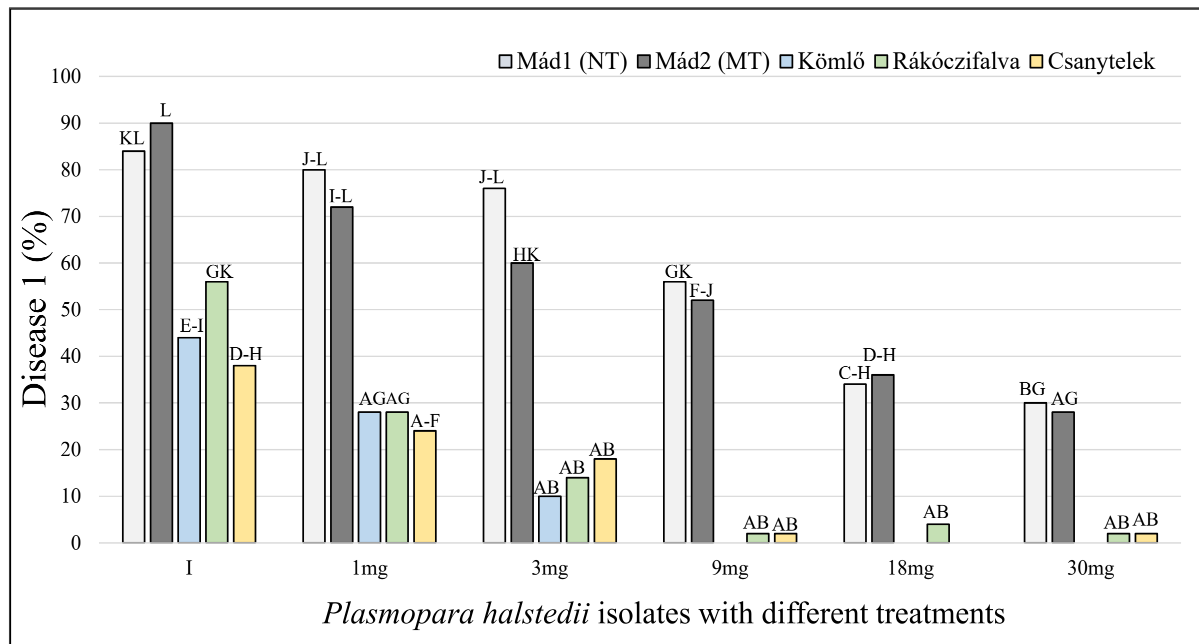
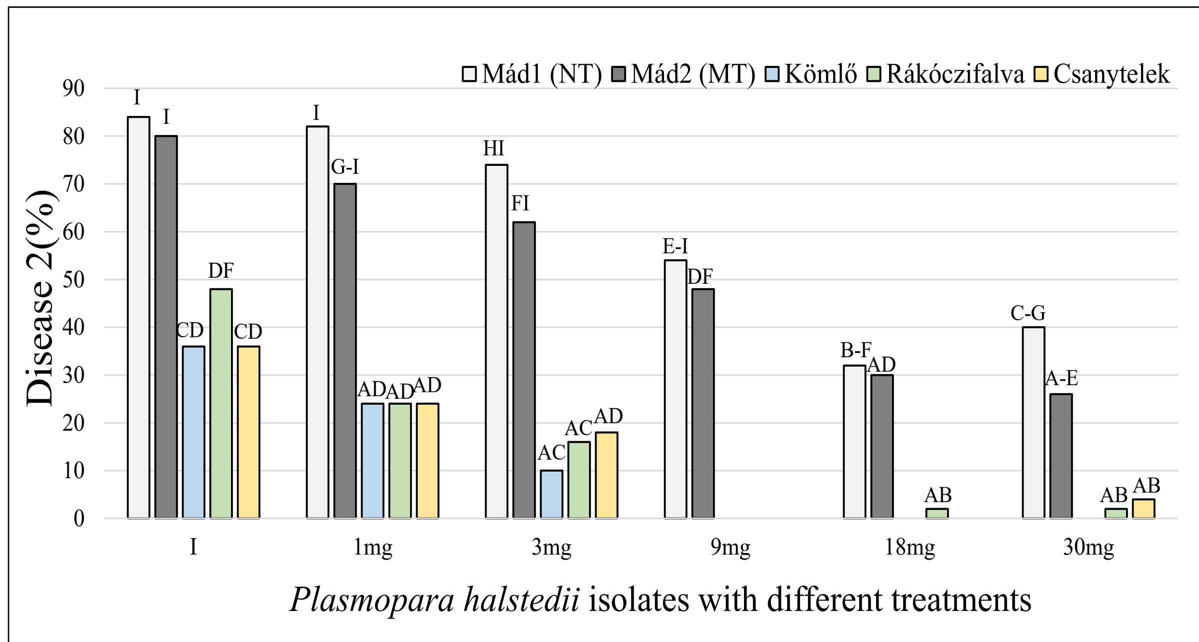


Figure 11. Disease 1 (%) values of sunflowers treated with different concentrations of mefenoxam and inoculated by 5 isolates of *Plasmopara halstedii* 9 days after inoculation.
The different letters displayed above the columns in the figure indicate a significant difference between treatments ($p < 0.05$).

1208 Mád1,2, Kömlő, Rákóczifalva, Csanytelek: codes of different *P. halstedii* isolates referring to their place
1209 of origin. Mád1 (NT) - the isolate from Mád was propagated (increased) on untreated plants with
1210 mefenoxam, Mád2 (MT) - the isolate from Mád was propagated (increased) on plants treated with
1211 mefenoxam at 3 mg/kg.
1212 I: control which was inoculated with *P. halstedii* sporangial suspension,
1213 1, 3, 9, 18, and 30 mg: treatment with different concentrations of mefenoxam (1, 3, 9, 18, and 30 mg/kg
1214 seeds) and inoculated with *P. halstedii* sporangial suspension
1215



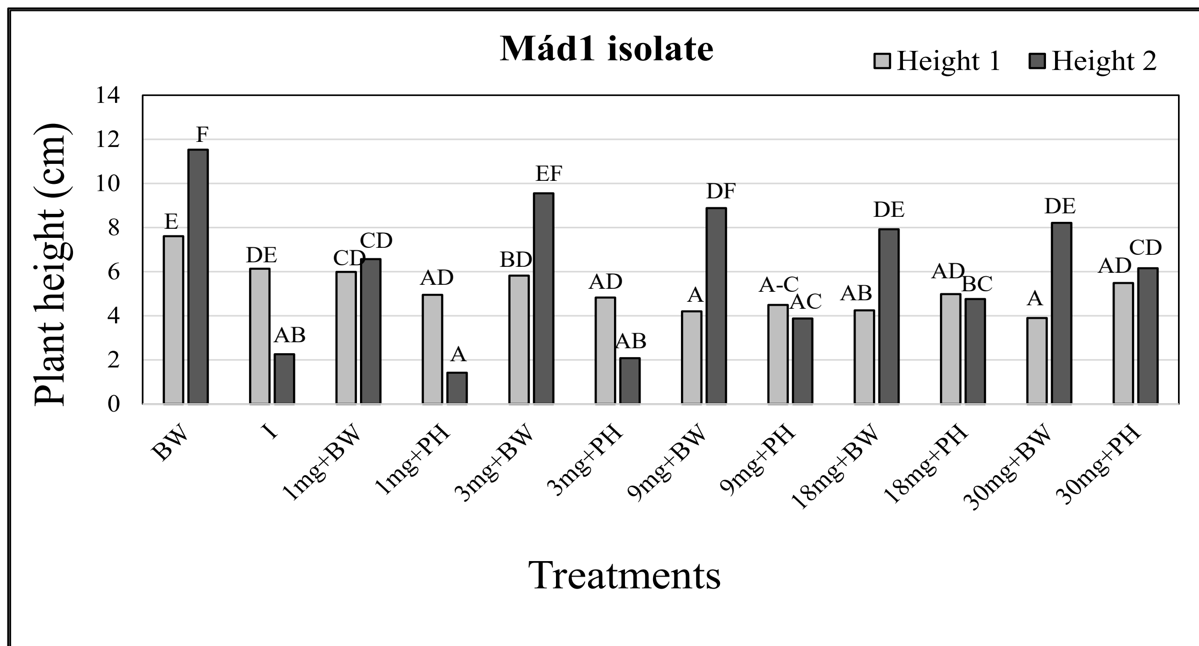
1216 **Figure 12.** Disease rates 2(%) on the sunflowers treated with different concentration of
1217 mefenoxam and inoculated by the different isolates of *Plasmopara halstedii* 21 days after
1218 inoculation.
1219 The different letters displayed above the columns in the figure indicate a significant difference between
1220 treatments (p <0.05).
1221 Mád1,2, Kömlő, Rákóczifalva, Csanytelek: codes of different *P. halstedii* isolates referring to their place
1222 of origin. Mád1 (NT) - the isolate from Mád was propagated (increased) on untreated plants with
1223 mefenoxam, Mád2 (MT) - the isolate from Mád was propagated (increased) on plants treated with
1224 mefenoxam at 3 mg/kg.
1225 I: control which was inoculated with *P. halstedii* sporangial suspension,
1226 1, 3, 9, 18, and 30 mg: treatment with different concentrations of mefenoxam (1, 3, 9, 18, and 30 mg/kg
1227 seeds) and inoculated with *P. halstedii* sporangial suspension.
1228

1229 **4.2.3.2 Plant heights**

1230 The stunting of the plants is a significant symptom of *P. halstedii*; hence, the plant height
1231 was measured twice for all isolates during the experiments.

1232 The heights of sunflowers connected to experiments with Mád 1 *P. halstedii* isolate are shown in
1233 Figure 13. After 9 days (Height 1), compared to control plants (BW), a significant height reduction
1234 was observed for the non-inoculated plants treated with different concentrations of mefenoxam.
1235 The situation was similar during the second evaluation (Height 2) except for non-inoculated plants
1236 treated with 3 and 9 mg/kg mefenoxam. In the case of the inoculated plants with Mád 1 isolate,
1237 there was no significant difference in Height 1 values between control (I) and treated plants in the

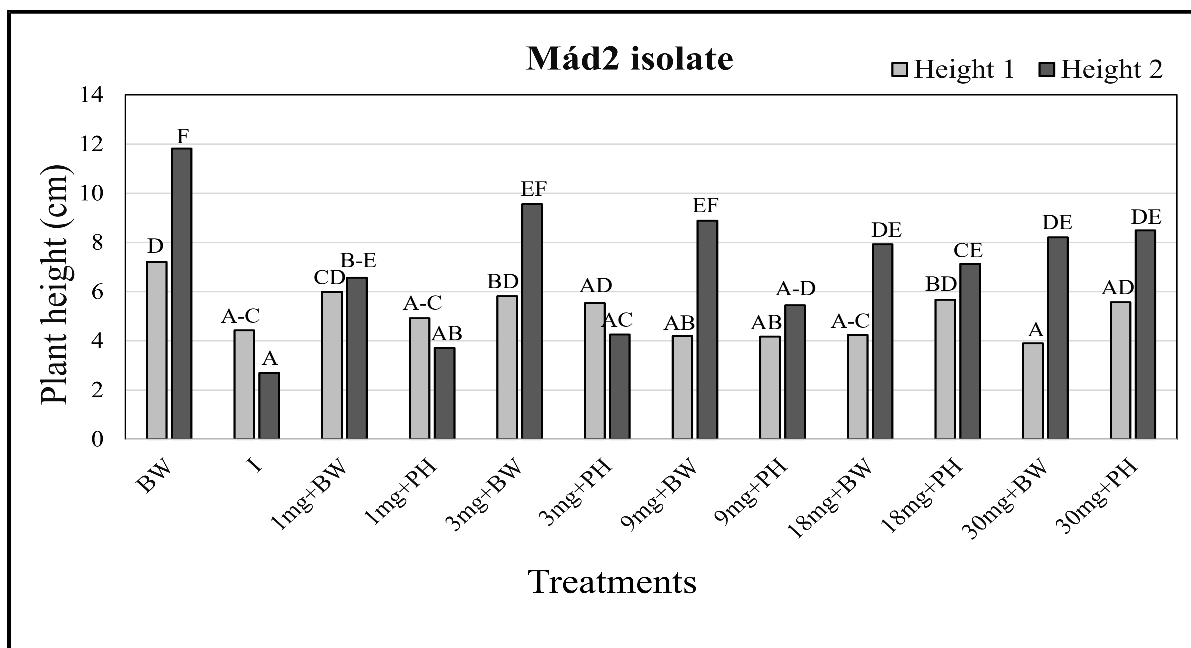
1238 first assessment. However, during the second assessment, only plants treated with 30 mg/kg
1239 mefenoxam could grow higher than the control.



1240
1241 **Figure 13.** Plant heights of sunflowers treated with different concentrations of mefenoxam
1242 inoculated with Mád1 isolate of *Plasmopara halstedii.*

1243 The different letters displayed above the columns in the figure indicate a significant difference between
1244 treatments ($p < 0.05$). BW: treated with bidistilled water and non-inoculated with *P. halstedii*, I: inoculated
1245 with *P. halstedii* sporangial suspension, 1, 3, 9, 18, and 30 mg: concentrations of mefenoxam, Height 1:
1246 height of sunflowers nine days after inoculation, Height 2: height of sunflowers 21 days after inoculation.
1247 The two height values (Height 1 and 2) are not statistically comparable in the figure.
1248
1249 The heights of sunflowers connected to experiments with Mád 2 *P. halstedii* isolate are shown in
1250 Figure 14. After 9 days (Height 1), compared to control plants (BW), a significant height reduction
1251 was observed for the non-inoculated plants treated with higher concentrations of mefenoxam (9,
1252 18, 30 mg). During the second evaluation of non-inoculated sunflowers (Height 2), plants treated
1253 with 1, 18, and 30 mg mefenoxam were significantly lower than the control plants. In the case of
1254 the inoculated plants with Mád 2 isolate, there was no significant difference in Height 1 values
1255 between control (I) and treated plants in the first assessment. However, similarly to Mád 1 isolate,
1256 during the second assessment, only plants treated with 18 and 30 mg/kg mefenoxam could grow
1257 higher than the control.

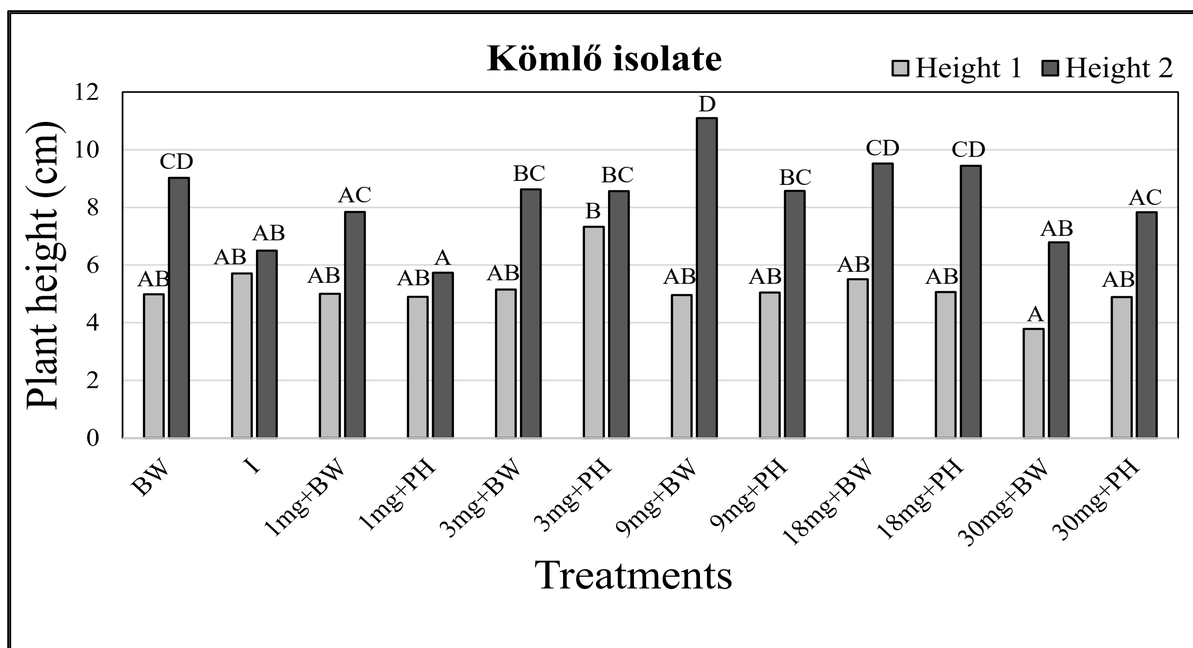
1258



1259 **Figure 14.** Plant heights of sunflower treated with different concentrations of mefenoxam
1260 inoculated with Mád2 isolate.

1261 The different letters displayed above the columns in the figure indicate a significant difference between
1262 treatments ($p < 0.05$). BW: treated with bidistilled water and non-inoculated with *P. halstedii*, I: inoculated
1263 with *P. halstedii* sporangial suspension, 1, 3, 9, 18, and 30 mg: concentrations of mefenoxam, Height 1:
1264 height of sunflowers nine days after inoculation, Height 2: height of sunflowers 21 days after inoculation.
1265 The two height values (Height 1 and 2) are not statistically comparable in the figure.
1266

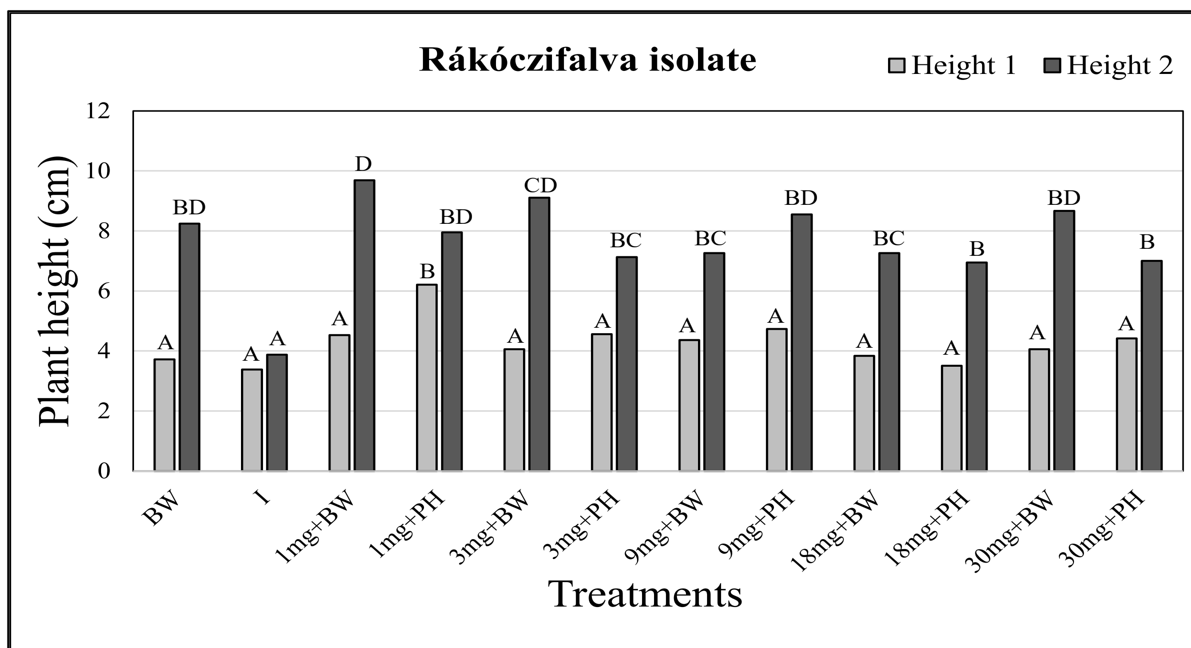
1267 The heights of sunflowers connected to experiments with Kömlő *P. halstedii* isolate are shown in
1268 Figure 15. There was no significant difference among the plant heights of non-inoculated
1269 sunflowers 9 days after inoculation (Height 1). During the second evaluation, only non-inoculated
1270 plants treated with 30 mg mefenoxam were significantly lower than non-treated ones. In the case
1271 of the inoculated plants with Kömlő isolate, there was no significant difference in Height 1 values
1272 between control (I) and treated plants in the first assessment. Twenty-one days after inoculation,
1273 it could be detected that inoculated plants treated with 18 mg mefenoxam were significantly higher
1274 than the control.



1275
1276 **Figure 15.** Plant heights of sunflower treated with different concentrations of mefenoxam
1277 inoculated with Kömlő isolate.

1278 The different letters displayed above the columns in the figure indicate a significant difference between
1279 treatments ($p < 0.05$). BW: treated with bidistilled water and non-inoculated with *P. halstedii*, I: inoculated
1280 with *P. halstedii* sporangial suspension, 1, 3, 9, 18, and 30 mg: concentrations of mefenoxam, Height 1:
1281 height of sunflowers nine days after inoculation, Height 2: height of sunflowers 21 days after inoculation.
1282 The two height values (Height 1 and 2) are not statistically comparable in the figure.
1283
1284 The heights of sunflowers connected to experiments with Rákóczifalva *P. halstedii* isolate are
1285 shown in Figure 16. There was no significant difference among the plant heights of non-inoculated
1286 sunflowers 9 and 21 days after inoculation (Height 1 and 2). During the first evaluation, no
1287 significant difference was observed among all the plant heights of inoculated sunflowers except
1288 plants treated with 1 mg mefenoxam, which were significantly higher. However, all treated and
1289 inoculated plants were significantly higher than the control 21 days after inoculation.
1290

1291

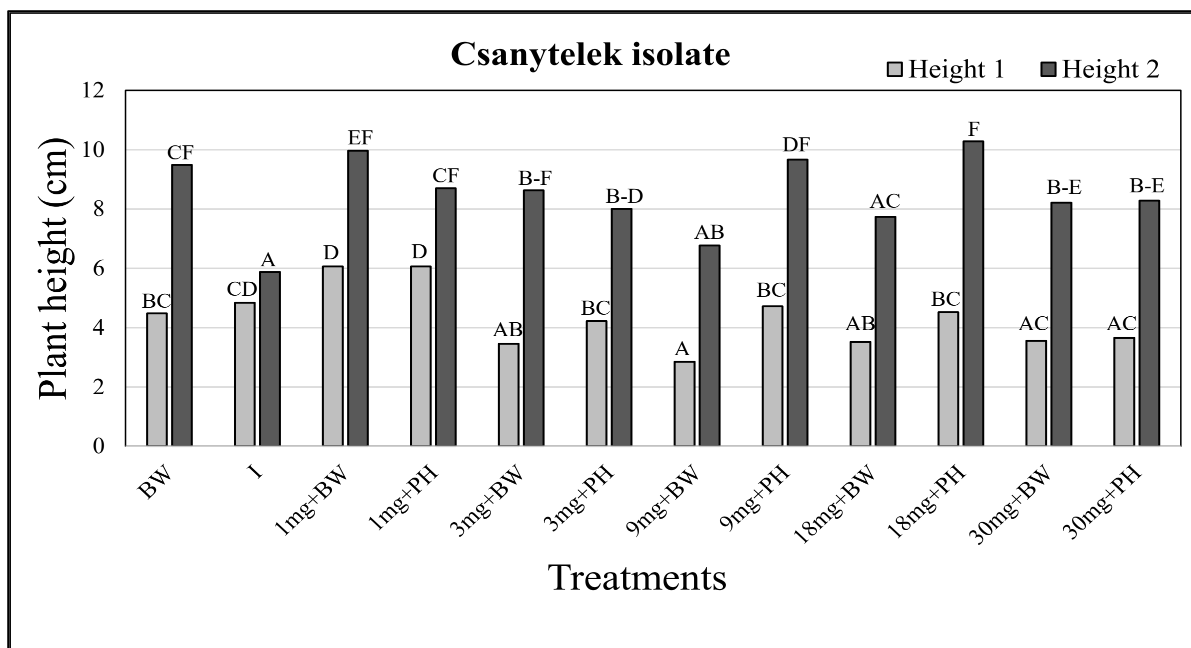


1292 **Figure 16.** Plant heights of sunflower treated with different concentrations of mefenoxam
1293 inoculated with Rákóczifalva isolate.

1294 The different letters displayed above the columns in the figure indicate a significant difference between
1295 treatments ($p < 0.05$). BW: treated with bidistilled water and non-inoculated with *P. halstedii*, I: inoculated
1296 with *P. halstedii* sporangial suspension, 1, 3, 9, 18, and 30 mg: concentrations of mefenoxam, Height 1:
1297 height of sunflowers nine days after inoculation, Height 2: height of sunflowers 21 days after inoculation.
1298 The two height values (Height 1 and 2) are not statistically comparable in the figure.

1299

1300 The heights of sunflowers connected to experiments with Csanytelek *P. halstedii* isolate are shown
1301 in Figure 17. There was no significant difference among the plant heights of non-inoculated
1302 sunflowers 9 and 21 days after inoculation (Height 1 and 2) except for plants treated with 1 and 9
1303 mg of mefenoxam. In the case of the inoculated plants with Csanytelek isolate, there was no
1304 significant difference in Height 1 values between control (I) and treated plants in the first
1305 assessment. However, twenty-one days after inoculation, it could be detected that all treated and
1306 inoculated plants were significantly higher than the control.



1307
1308 **Figure 17.** Plant heights of sunflower treated with different concentrations of mefenoxam
1309 inoculated with Csanytelek isolate.

1310 The different letters displayed above the columns in the figure indicate a significant difference between
1311 treatments ($p < 0.05$). BW: treated with bidistilled water and non-inoculated with *P. halstedii*, I: inoculated
1312 with *P. halstedii* sporangial suspension, 1, 3, 9, 18, and 30 mg: concentrations of mefenoxam, Height 1:
1313 height of sunflowers nine days after inoculation, Height 2: height of sunflowers 21 days after inoculation.

1314 The two height values (Height 1 and 2) are not statistically comparable in the figure.

1315

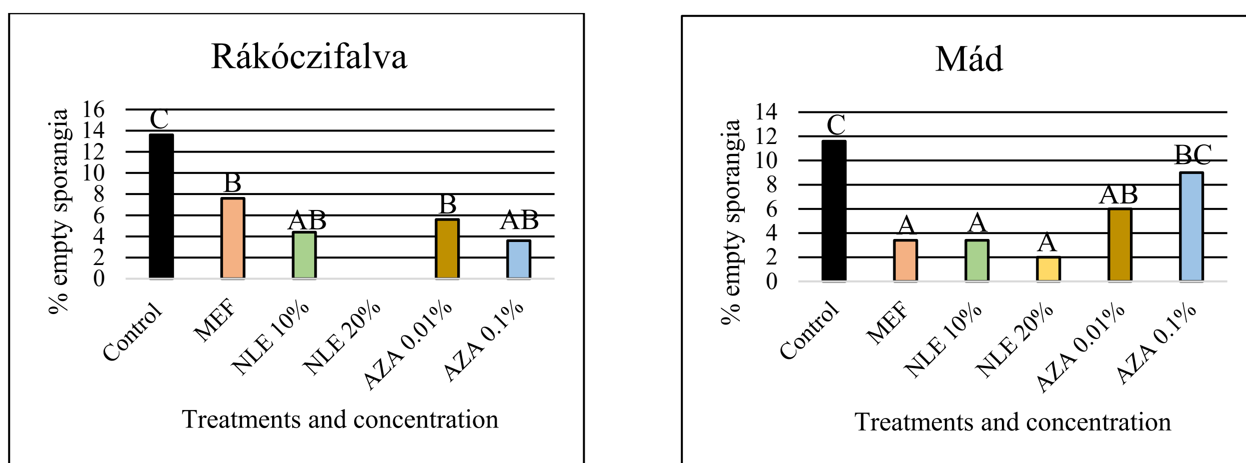
1316 **4.3 Effects of neem-derived pesticides on sunflower downy mildew**

1317 **4.3.1 *In-vitro* experiment: Examination of the effect of neem-derived pesticides on *P. halstedii***
1318 **sporangial germination**

1319 The microscopical examination of sporangia was done 24 h after treatment with neem leaf extract
1320 and NeemAzal T/S (1% azadirachtin). For the Rákócifalva isolate, the statistical analysis showed
1321 that all the neem-derived pesticide treatments significantly decreased the number of empty
1322 sporangia, thus inhibiting germination. For the Mád isolate, all the treatments, except AZA 0.1%,
1323 were found to be significantly better than the control (no treatment) at reducing the number of
1324 empty sporangia (which includes completely or partially empty sporangia, Figure 18).

1325

1326



1327
1328 **Figure 18.** Effect of two different concentrations of neem leaf extract (NLE) and NeemAzal T/S
1329 (AZA), respectively, on the germination of *P. halstedii* sporangia of two isolates (Rákóczifalva
1330 and Mád). Mefenoxam (MEF) was used as a positive control.

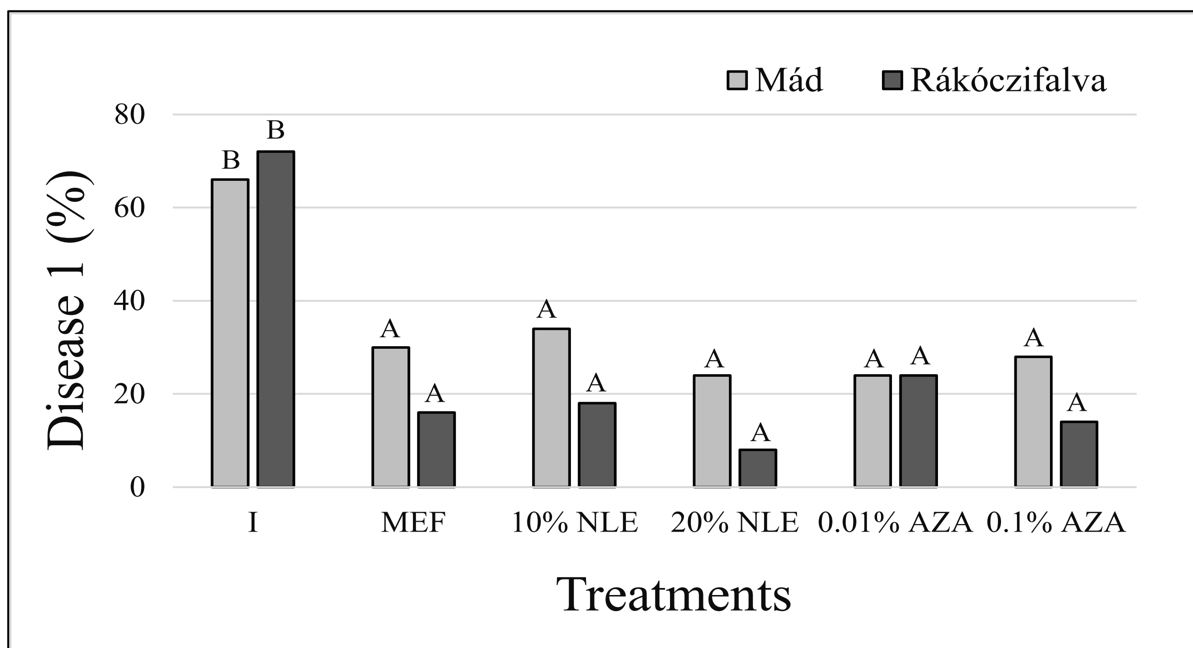
1331 Different letters according to Tukey's test indicate significant difference at 95% confidence level.
1332

1333 4.3.2 Pre-treatment effect of neem-derived pesticides on *Plasmopara halstedii* isolates in *in-* 1334 *vivo* conditions

1335 4.3.2.1 Neem effects on disease rates and plant heights

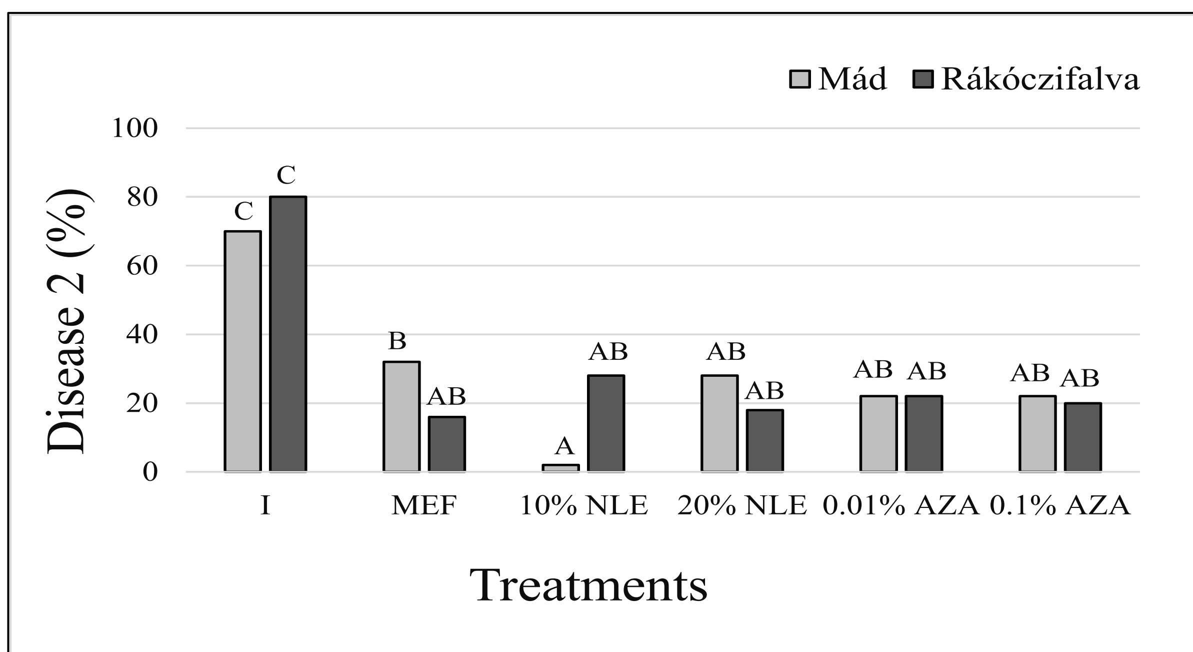
1336 On the two assessment dates, both concentrations of neem leaf extract and NeemAzal T/S and
1337 mefenoxam treatment were found to reduce the sporulation of *P. halstedii* isolates Mád and
1338 Rákóczifalva significantly (Figures 19 and 20). Thus, the pre-treatments significantly affected the
1339 sporulation, pre-damping-off, chlorosis, and post-damping-off caused by *P. halstedii*.

1340 The plant heights for first and second evaluation are shown in Figures 21 and 22. Plant heights of
1341 non-inoculated sunflowers were similar all over this experiment. The plant heights of inoculated
1342 sunflowers with Mád isolate were significantly higher than that of the control for the mefenoxam
1343 treated and lower for the NLE10% treated plants in the first evaluation. The plant heights of
1344 inoculated sunflowers with the Rákócifalva isolate were significantly higher than that of the
1345 control for the AZA0.01 treated plants in the first evaluation. During the second evaluation, the
1346 heights of inoculated and treated plants with different neem-products were significantly higher
1347 than the control (except NLE10% Mád and AZA0.01 Rákóczifalva treatments).

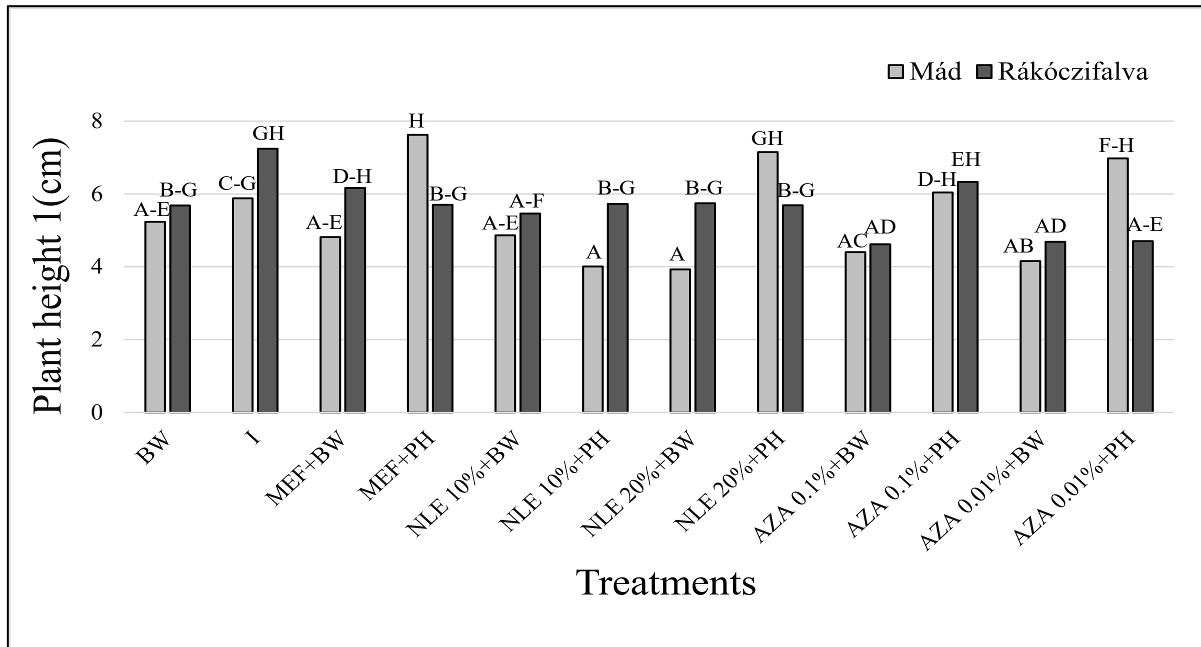


1348
1349 **Figure 19.** Disease 1 (%) of the sunflowers treated with neem leaf extract (NLE) and NeemAzal
1350 T/S (AZA) after 9 days inoculation.

1351 I; non-treated with mefenoxam and inoculated with *P. halstedii*, MEF; treated – treated with mefenoxam
1352 (3 mg/kg seed) and inoculated with *P. halstedii*, 10% NLE; 20% NLE; 0.01% AZA; 0.1% AZA; inoculated
1353 with *P. halstedii*. Bars sharing the same letter are not significantly different.

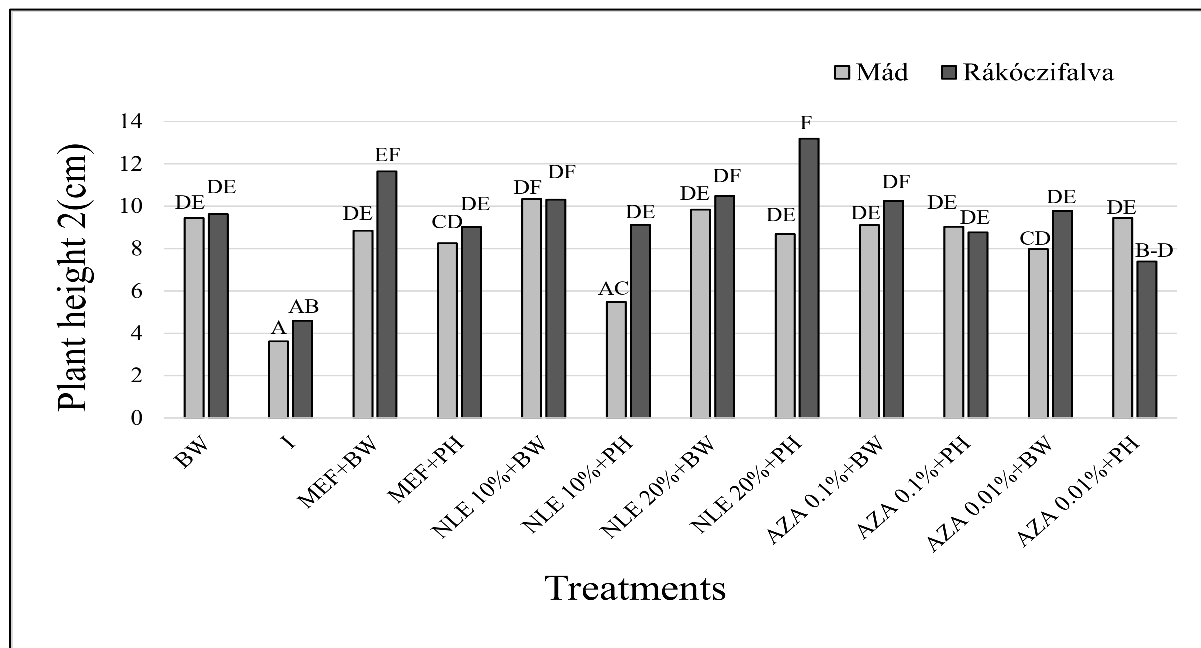


1354
1355 **Figure 20.** Disease 2 (%) on the sunflowers treated with the concentration of neem leaf extract
1356 and NeemAzal T/S (AZA) after 21 days inoculation.
1357
1358 I; non-treated with mefenoxam and inoculated with *P. halstedii*, MEF; treated – treated with mefenoxam
1359 (3 mg/kg seed) and inoculated with *P. halstedii*, 10% NLE; 20% NLE; 0.01% AZA; 0.1% AZA; inoculated
1360 with *P. halstedii*. Bars sharing the same letter are not significantly different.



1361 **Figure 21.** Plant heights of the neem leaf extract and NeemAzal T/S (AZA) treated sunflowers 9
1362 days after inoculation with *P. halstedii.*

1363 BW: treated with bidistilled water, I: non-treated with mefenoxam and inoculated with *P. halstedii*,
1364 MEF+BW: treated with mefenoxam (3 mg/kg seed) and bidistilled water, MEF+PH: treated with
1365 mefenoxam and inoculated with *P. halstedii,* 10% NLE; 20% NLE; 0.01% AZA; 0.1% AZA: treated with
1366 different concentrations of neem leaf extract or azadirachtin and inoculated with *P. halstedii*. Mád,
1367 Rákóczifalva: isolates of *P. halstedii*. Bars sharing the same letter are not significantly different.



1368
1369 **Figure 22.** Plant heights of the neem leaf extract and NeemAzal T/S (AZA) treated sunflowers
1370 21 days after inoculation with *P. halstedii.*

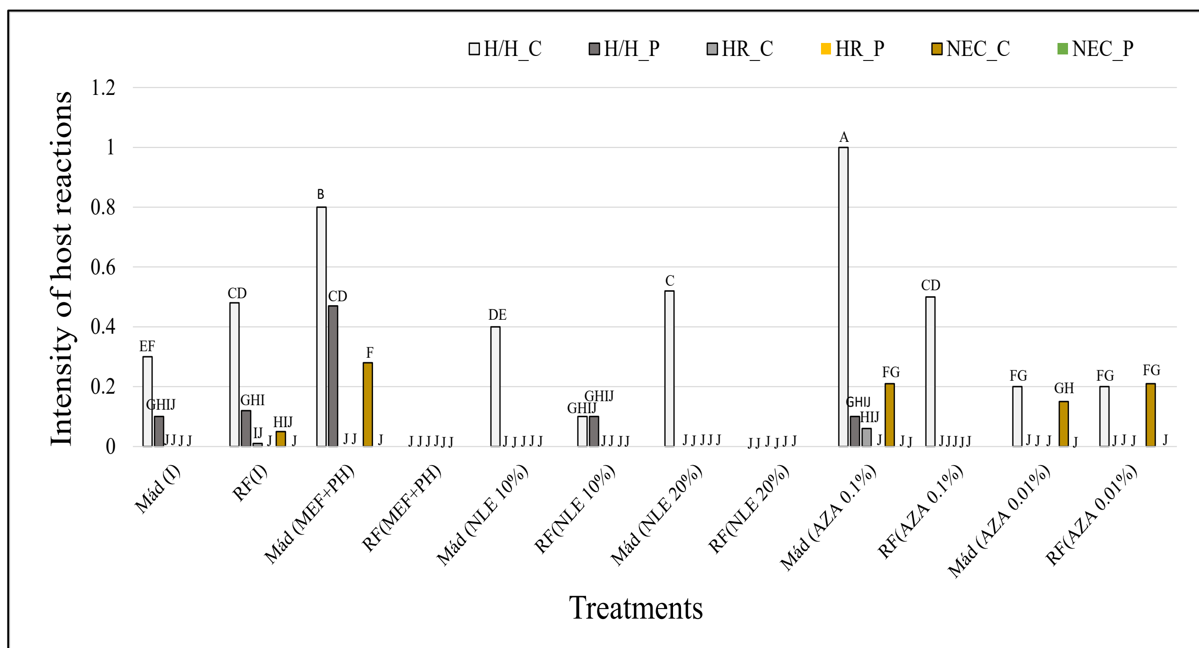
1371 BW: treated with bidistilled water, I: non-treated with mefenoxam and inoculated with *P. halstedii*,
1372 MEF+BW: treated with mefenoxam (3 mg/kg seed) and bidistilled water, MEF+PH: treated with
1373 mefenoxam and inoculated with *P. halstedii,* 10% NLE; 20% NLE; 0.01% AZA; 0.1% AZA: treated with

1374 different concentrations of neem leaf extract or azadirachtin and inoculated with *P. halstedii*. Mád,
1375 Rákóczifalva: isolates of *P. halstedii*. Bars sharing the same letter are not significantly different.

1376 **4.3.2.2 Microscopic observations**

1377 The rate of pathogen hyphae and host reactions, such as hypersensitive reaction and necrosis are
1378 shown in Figure 23. Intercellular hyphae and haustoria were able to spread in the cortical and pith
1379 parenchyma of most treated and non-treated plants inoculated with different *P. halstedii* isolates.
1380 In addition, in plants treated with mefenoxam and inoculated with the Mád isolate, hyphae of the
1381 pathogen were significantly more abundant both in the cortical and pith parenchyma than that of
1382 the non-treated plants. The opposite was true for the mefenoxam-treated plants inoculated by the
1383 Rákóczifalva isolate.

1384 In sunflowers treated with Neem-derived pesticides and inoculated by Mád isolate significantly
1385 more hypha could be observed compared to non-treated ones in the cortical than in the pith
1386 parenchyma. No hypersensitive reaction and necrosis could be detected in the pith parenchyma
1387 for all treatments in both isolates. More necrosis in the cortical parenchyma cells was observed in
1388 AZA 0.01% treated sunflowers than in inoculated controls for Rákóczifalva isolate. Similarly, we
1389 could detect more necrosis in the cortical part of sunflowers treated with both concentrations of
1390 AZA and mefenoxam inoculated by the Mád isolate.



1391
1392 **Figure 23.** Effects of neem leaf extract (NLE), NeemAzal T/S (AZA) and mefenoxam (MEF) on
1393 the occurrence of the pathogen hypha and haustoria (H/H) and host tissue responses such as
1394 hypersensitive reaction (HR) and necrosis (NEC) in the cortical (C) and pith (P) parenchyma
1395 inoculated with *Plasmopara halstedii* isolates (Mád and RF: Rákóczifalva).

1396 I: stands for inoculated, non-treated control. Bars sharing the same letter are not significantly different.
1397

1398

5. DISCUSSION

5.1 Pathotype composition of sunflower downy mildew (*Plasmopara halstedii*) in Hungary

Downy mildew of sunflower is one of the most widespread diseases caused by *P. halstedii* and affects the crop worldwide. Moreover, *P. halstedii* has several pathotypes with varying degrees of virulence. Therefore, knowing the virulence diversity within the pathogen population of sunflower downy mildew has become essential for resistance breeding and quarantine measures. Thus, the background knowledge of the distribution of *P. halstedii* pathotypes is of utmost importance for effective pest management (Virányi et al. 2015, Spring 2019, Bán et al. 2021, Miranda-Fuentes et al. 2021).

The differentiation of the pathogen started in the 1970s when the first resistance gene was implemented in the sunflower line RHA266 (Vranceanu and Stoenescu 1970, Spring 2019). The acceleration of the process has made it necessary to develop a protocol to standardize the pathotyping process. Today the pathotype characterization of *P. halstedii* is based on an internationally accepted methodology with 9 sunflower differential lines to serve as a standard method worldwide (Trojanová et al. 2017). Although several attempts have been made to develop modern methods for this purpose, they have not yet been widely used in practice due to their many drawbacks (Gascuel et al. 2016). Therefore, we decided to use the traditional and widely accepted method of Trojanová et al. (2017) for pathotype identification.

Gulya (2007), Virányi et al. (2015), and Spring (2019) have previously summarized the pathotype distribution of *P. halstedii* worldwide. Recently, it has been found that several pathotypes have overcome the *Pl6* resistance gene incorporated into a wide range of sunflower hybrids and led to the emergence of highly aggressive pathotypes (Sedlárová et al. 2016, García-Carneros and Molinero-Ruiz 2017, Bán et al. 2018, Spring and Zipper 2018). Moreover, Martin-Sanz et al. (2020) reported a virulent pathotype 714, which has overcome to *Pl8* resistance gene.

Before 2010, Gulya (2007) reported about five pathotypes (100, 330, 700, 710, and 730) considered relevant in Hungary. There was a significant change in the virulence character of *P. halstedii* populations detected between 2007 and 2013 in Hungary and worldwide. However, despite new pathotypes, less virulent pathotypes such as 700 and 730 were still predominant in Hungary from 2007 to 2014 (Virányi et al. 2015).

More recently, Bán et al. (2021) updated the distribution of pathotypes of sunflower downy mildew in seven European countries and reported 18 new pathotypes in six countries. This dissertation is part of this work, which presents data for Hungary from 2017 to 2019. As a result, besides the

1432 dominance of high virulent pathotypes such as 704, the presence of less virulent pathotypes (700
1433 and 730) was also confirmed in our study from 2017 to 2019. In addition, we identified pathotype
1434 734 for the first time in Hungary (and Central Europe) during this period (Nisha et al. 2021). This
1435 pathotype is likely widespread in Hungary because it was detected from three different regions of
1436 the country: Borsod-Abaúj-Zemplén county (Léh), Békés county (Bucsa, Kertészsziget) and
1437 Nógrád county (Vanyarc). The possibility that pathotype 734 is present in Hungary has been raised
1438 before (Iwebor et al. 2018), but previous isolates were proved weak for proper identification. This
1439 pathotype has already been widespread among hot races in the USA and Russia (Spring 2019) and
1440 is considered highly aggressive, which was able to overcome the effect of resistance genes *Pl6*
1441 and *Pl7*.

1442 Previously the occurrence of the globally new pathotype, 724, has been reported only in Hungary,
1443 from two regions in Békés county (Mezőkovácsháza and Szeghalom) (Bán et al. 2018). Later,
1444 pathotype 724 was also detected in Romanian samples in 2019 (Bán et al. 2021). In this work, we
1445 confirmed its presence in two more sites in Békés county (Békésszentandrás and Vésztő). In
1446 addition, further spread into the western part of the country was proved as we identified the
1447 pathotype 724 in Bonyhád (Tolna county). This fact is noteworthy because no highly virulent
1448 pathotypes have been reported from the western part of Hungary so far. Moreover, the only data
1449 available in this part of the country on sunflower downy mildew pathotypes are from Martonvásár
1450 (pathotype 700).

1451 It is remarkable that, to a smaller extent, less virulent pathotypes such as 700 and 730 could be
1452 identified from sunflowers with resistance genes against these strains during our survey. The exact
1453 reason for this is still unknown, but other authors report similar cases for different pathogens
1454 (Kema et al. 2018, Seybold et al. 2020). A highly aggressive pathotype likely represses the host's
1455 defense mechanisms, creating favorable conditions for the less virulent (or avirulent) pathotypes.
1456 It is even likely that lower virulence in these strains is associated with higher fitness, contributing
1457 to their persistence.

1458 Finally, this work supports the previous considerations by Virányi et al. (2015) that there is a shift
1459 in the pathotype composition of sunflower downy mildew in Hungary. However, many more
1460 samples and frequent sampling would be needed to prove this pathogenic shift.

1461 Several factors may be responsible for the emergence of new *P. halstedii* pathotypes such as
1462 favorable weather conditions, the emergence of mefenoxam-resistant *P. halstedii* isolates (Körösi
1463 et al. 2020), and the spread of minimal tillage systems. In Hungary, however, mainly short crop

1464 rotation and, in many cases, inadequate weed management may promote the distribution of new
1465 aggressive *P. halstedii* pathotypes (Bán et al. 2016, 2021).

1466 **5.2 Mefenoxam-sensitivity of Hungarian *P. halstedii* isolates**

1467 The widespread use of mefenoxam has resulted in a decline in efficacy against sunflower downy
1468 mildew in some western European countries (Lafon et al. 1996; Albourie et al. 1998, Molinero-
1469 Ruiz et al. 2003) and the USA (Gulya 2000). Some data supported by greenhouse experiments
1470 were available in Hungary by Oros and Virányi (1984), but they could not prove it in further tests
1471 with field isolates of *P. halstedii* (Virányi and Walcz 2000). Similarly, resistance to mefenoxam
1472 has already been reported in other populations of different oomycetes (Schwinn and Staub 1987,
1473 Lamour and Hausbeck 2000, Parra and Ristaino 2001,).

1474 In our first fungicide resistance study, 10 isolates of *P. halstedii* were tested using the WSI (whole
1475 seedling immersion) method. Here, we were interested in how the registered rate of mefenoxam
1476 (3mg/kg seed) influences the development of initial symptoms and signs (sporulation, early
1477 damping-off, decrease in plant height) of different *P. halstedii* isolates originated mainly from
1478 hybrids where mefenoxam was applied as a seed coating. Mefenoxam performed poorly or only
1479 moderately in the case of half of the examined *P. halstedii* isolates in our test. Although a limited
1480 number of samples have been analyzed, these results provide the first evidence of mefenoxam
1481 tolerance of sunflower downy mildew in high oleic sunflower hybrids in Hungary. Furthermore,
1482 like Gulya (2000), our results did not find any correlation between the virulence phenotype (CVF)
1483 and the fungicide resistance characteristic of different *P. halstedii* strains, i.e., there were also
1484 sensitive and resistant strains characterized by either the 704 or 724 pathotypes.

1485 Continuing the sensitivity studies with additional *P. halstedii* isolates, the SDI (soil drench
1486 inoculation) method was used in the next series of 8 isolates, which better models the natural
1487 infection of the pathogen. The development of subsequent symptoms (e.g., leaf chlorosis, late
1488 damping-off) caused by the pathogen was monitored in addition to the initial symptoms. We were
1489 also curious to see if there were differences in plant responses such as hypersensitive reaction and
1490 cell necrosis in plants infected with isolates of different sensitivities. We performed detailed
1491 statistical analyses here to show differences.

1492 Both mefenoxam-treated and non-treated plants formed two relatively distinct groups (clusters)
1493 based on the cluster analysis of disease rates and plant heights in the 8 isolate experiment. The
1494 sunflowers in Cluster 1 (non-treated and inoculated by isolates 5, 6, 9, 11) had relatively high
1495 initial and subsequent infection rates, indicating that the pathogen could penetrate unhindered into
1496 the upper parts of the plant. This was associated with significant growth inhibition of these plants.

1497 On the other hand, the reaction was similar in mefenoxam-treated plants inoculated by isolate 11;
1498 therefore, it appears to be mefenoxam resistant. Although this is a typical reaction of susceptible
1499 sunflowers to the pathogen, it is interesting that in Cluster 2, non-treated plants (inoculated with
1500 isolates 1, 4, 7, 8) were characterized by the decreased spreading of the pathogen to the above
1501 plant parts. This difference between the two clusters (mainly non-treated plants) can probably be
1502 explained by the different aggressiveness of the *P. halstedii* isolates tested as indicated by other
1503 authors (e.g., Sakr 2009). Nevertheless, the two clusters of mefenoxam-treated and inoculated
1504 plants (Clusters 3 and 4) also differed, mainly in the degree of initial disease rate and in the
1505 development of plant heights. In conclusion, treatment with mefenoxam had different effects on
1506 different *P. halstedii* isolates, according to disease rates and plant heights.

1507 Pearson correlation, especially during the second evaluation, showed a strong negative correlation
1508 between the disease rate and plant height values of both non-treated and treated plants. This
1509 negative correlation is not surprising, as many authors have reported such effects of the pathogen
1510 on plant development in susceptible, non-treated sunflowers (Virányi and Oros 1991, Gascuel et
1511 al. 2015). In the case of treated plants, this negative correlation is presumably related to fungicide
1512 tolerance (resistance) since if the pathogen can spread within the plant, the growth-reducing effect
1513 is exerted.

1514 Host responses of sunflowers (susceptible, resistant) inoculated with *P. halstedii* have already
1515 been examined by several authors (Allard 1978, Wehtje et al. 1979, Gray and Sackston 1985,
1516 Mouzeyar et al. 1993, 1994, Bán et al. 2004, Radwan et al. 2011). Mouzeyar et al. (1993, 1994)
1517 pointed out that *P. halstedii* could infect susceptible and resistant sunflower lines in a microscopic
1518 investigation. Although to a smaller extent, even a susceptible plant can react to the pathogen's
1519 spread. Our results with fluorescent microscopy of non-treated sunflowers also supported this.

1520 Moreover, the speed and intensity of host tissue response to *P. halstedii* in a resistant sunflower
1521 may vary, and it can appear in the root or different parts of the hypocotyl (Mouzeyar et al. 1993).
1522 Previous authors also described a hypersensitive-like response in the hypocotyl of mefenoxam-
1523 treated susceptible sunflowers (Mouzeyar et al. 1995). They found that all metalaxyl
1524 concentrations and application modes provided complete protection against *P. halstedii*. However,
1525 only one *P. halstedii* isolate was tested in the latter work, which seemed sensitive to the active
1526 ingredient.

1527 We first revealed a clear difference in host tissue responses of mefenoxam-treated susceptible
1528 sunflowers inoculated with various *P. halstedii* isolates. Treated sunflowers inoculated by some
1529 isolates (6, 8, and 11) showed hyphal growth in the cortical and pith parenchyma. The cortical part

1530 could also detect a moderate hypersensitive reaction and necrosis. This phenomenon was very
1531 similar to what usually occurs in non-treated susceptible plants with the plant response appearing
1532 to be a delayed host reaction to a pathogenic attack (Mouzeyar et al. 1993, Gascuel et al. 2015).
1533 For other *P. halstedii* isolates, we could detect a limited or no mycelial growth in the mefenoxam-
1534 treated plants, which was accompanied by weak or no reactions of treated sunflowers in their
1535 hypocotyls. Because of the lack of massive mycelial growth in the hypocotyl, it is likely, that the
1536 pathogen was arrested in the root tissues by the chemical.

1537 In our 8 isolate study, cluster analyses of sunflowers based on the microscopic variables showed
1538 clear differentiation of three groups of mefenoxam-treated sunflowers inoculated by different *P.*
1539 *halstedii* isolates. Those in the first two groups (clusters) showed increased (isolates 8 and 11) or
1540 moderate tolerance (isolate 6) to mefenoxam, while isolates in the third group showed sensitivity.
1541 Disease rate and plant height values of treated and inoculated sunflowers with these tolerant or
1542 resistant isolates also supported this. However, microscopic studies allowed us to estimate the
1543 sensitivity (tolerance) more accurately, showing refined interaction with non-treated plants. In
1544 addition, only isolate 11 could be defined with more decreased sensitivity with the evaluation of
1545 visible symptoms.

1546 In addition to its direct toxic effect on the pathogen, metalaxyl (mefenoxam) activates the host
1547 defense system, which might result in increased sunflower resistance, restricting pathogen
1548 development (Cahill et al. 1993). In previous research, histological alterations such as haustoria
1549 encapsulation by callose deposits (Hickey and Coffey 1980) or the development of limited
1550 hypersensitive-like lesions were also reported, followed by metalaxyl treatment in some host-
1551 parasite interactions where the pathogen was sensitive to the chemical (Ward et al. 1980,
1552 Lazarovits and Ward 1982, Stössel et al. 1982, Mouzeyar et al. 1995). However, the question
1553 remains whether the direct (fungistatic) or indirect effect (through the host) of metalaxyl is more
1554 significant against the sensitive pathogen in different host-parasite relationships.

1555 Examining metalaxyl-sensitive and tolerant *Phytophthora megasperma* isolates in soybean Cahill
1556 and Ward (1989) pointed out that metalaxyl enhanced the release of phytoalexin elicitors
1557 (glyceollin) in culture fluids of the sensitive isolate but not in those of the tolerant isolate.
1558 Releasing elicitors due to metalaxyl treatment could induce host reactions in compatible
1559 interactions with the sensitive isolate. In our study, the effective host tissue responses against the
1560 sensitive *P. halstedii* isolates likely occurred at a very early stage of infection in the roots of
1561 mefenoxam-treated sunflowers. Despite this, the reaction of mefenoxam-treated plants to tolerant
1562 isolates could appear later in the hypocotyl, which the delayed stimulation of elicitor activity can
1563 explain by the chemical. Our results with the Spearman correlation also demonstrate this. It

1564 showed that the spread of the tolerant isolates in the cortical parenchyma of treated plants
1565 correlated positively with the appearance of HR and necrosis.

1566 Interestingly, more abundant hyphae were found in the pith of treated than non-treated plants
1567 inoculated by isolates 8 and 11 (considered as tolerant or resistant). This is in line with the results
1568 of Cahill and Ward (1989). They reported a better growth of metalaxyl-tolerant *Phytophthora*
1569 *megasperma* isolates in the presence of the chemical *in vitro* and *in vivo*. Previous authors assumed
1570 that metalaxyl could serve as a nutrient and raised the idea of other tolerance mechanisms and
1571 different interactions with the host (soybean) for those tolerant isolates. In addition, the more
1572 significant presence of the pathogen in the pith of sunflowers has been shown to facilitate the
1573 spread of the pathogen to the upper parts of the plant (e.g., epicotyl) (Heller et al. 1997).

1574 Further studies are needed to explore the reasons for the differences in tissue responses to sensitive
1575 and tolerant isolates of *P. halstedii* in sunflower. In addition, how plant defense mechanism
1576 contributes to the effectiveness of fungicides also has to be elucidated.

1577 In the third part of the fungicide sensitivity studies, the effects of different mefenoxam
1578 concentrations were tested against 5 isolates of *P. halstedii* with the WSI method for inoculation.
1579 One isolate (Mád) was propagated on both non-treated (Mád1) and mefenoxam treated plants
1580 (Mád2); the others could only be propagated on non-treated plants. Although EC50 values were
1581 not established, we estimated the mefenoxam concentration (interval) at which at least half of the
1582 plants showed symptoms and signs of *P. halstedii*. These values varied, ranging from 18 to 30 mg
1583 for two isolates (Mád1 and 2) and from 1 to 3 mg for the others. On this basis, the Mád isolate is
1584 further evidence of the presence of mefenoxam tolerance/resistance in Hungary, as it was only
1585 effective against it at several folds of the registered concentration (3 mg/kg seeds).

1586 **5.3 Efficacy of neem-derived pesticides to restrict sunflower downy mildew**

1587 The effect of two different neem-derived pesticides, such as neem leaf extract (NLE) and
1588 NeemAzal T/S, was tested in different concentrations for the first time against *P. halstedii in*
1589 *vitro* and *in vivo* conditions by Doshi et al. (2020). The authors reported that those neem-derived
1590 pesticides could be valuable for controlling the downy mildew of sunflower, but only one *P.*
1591 *halstedii* isolate was used in those studies, which was sensitive to mefenoxam. Previously, Mirza
1592 et al. (2000) tested the neem products on *Phytophthora infestans in vitro*. They reported the
1593 effectiveness of all the products, namely crude neem seed oil, nimbokil (a commercial formulation
1594 of neem oil), crude terpenoid extract of neem seed oil, and neem leaf decoction against mycelial
1595 growth, sporangial germination, and sporangium production of *Ph. infestans*. It was shown that all
1596 these products could potentially manage potato late blight disease. Similarly, Rashid et al. (2004)

also observed that all the neem products significantly inhibited the different developmental stages of the above pathogen.

We tested neem leaf extracts (NLE) and azadirachtin (AZA as NeemAzal T/S) in different concentrations against two different *P. halstedii* isolates by treating plants with the ingredients for a longer exposure time (4 h) than Doshi et al. (2020). In addition, we studied host tissue responses (hypersensitive reaction and cell necrosis) of neem-treated, inoculated plants with a fluorescent microscope to explore the histological background of protection.

Under *in vitro* conditions, all the treatments except the higher concentration of NLE showed significant inhibition of the sporangial germination of Rákóczifalva isolate. Similarly, except for AZA 0.1%, all the treatments were significantly better than the inoculated control at reducing the total number of empty sporangia for Mád isolate.

When tested *in vivo* in our study, both concentrations of neem leaf extracts and NeemAzal T/S significantly reduced the sporulation and chlorosis of *P. halstedii* isolates as compared to inoculated control plants. Our results were consistent with the findings of Achimu and Schlösser (1992), where neem seed extract and commercial neem products were effective against *Plasmopara viticola* in the grapevine. Similarly, Krzyzaniak et al. (2018) also found that the plant extract successfully controlled *P. viticola*. The reduction of infection in the pre-treatment may be due to sunflower sensitizing defense response against *P. halstedii*, reported by Fernandez et al. (2004), where they tested the essential oil obtained from *Bupleurum gibraltarium* against the pathogen. They reported that pre-treatment with oil might activate the defense response of the seedlings against *P. halstedii*.

Host tissue responses of neem-treated sunflowers inoculated by *P. halstedii* were first examined by fluorescent microscope in our study. We observed a similar tissue response (cell necrosis) in neem-treated and inoculated plants as previously observed in BTH (benzothiadiazole as Bion 50WG) treatments against sunflower downy mildew (Bán et al. 2004) and sclerotinia (Bán et al. 2017). Similarly, we could detect more necrosis in the cortical part of sunflowers treated with both concentrations of AZA and mefenoxam inoculated by the Mád isolate as compared to the non-treated control. This was true for plants treated with 0.01 % AZA and inoculated with the Rákóczifalva isolate. Therefore, it seems that azadirachtin induces similar host tissue responses in diseased plants to mefenoxam and benzothiadiazole, which can play a role in restricting *P. halstedii* in susceptible sunflowers.

1629 # 6. CONCLUSIONS AND RECOMMENDATIONS

1630 The high variability of *P. halstedii* is an important trait of the pathogen allowing it to overcome
1631 the resistance genes and the effectiveness of the compounds such as mefenoxam. Therefore, the
1632 key task and goal of the future research is to monitor the pathotype composition and fungicide
1633 resistance of the pathogen. This facilitates the efficient resistance breeding and the development
1634 of new active substances against the pathogen in order to get good quality and produce high yields.
1635 Also, the broader use of integrated plant protection could significantly slow down the evolution of
1636 new pathotypes of *P. halstedii*.

1637 *Plasmopara halstedii* has several pathotypes with varying degrees of virulence. Recently, highly
1638 aggressive pathotypes have emerged worldwide. Information on the virulence diversity within the
1639 population of sunflower downy mildew become essential for resistance breeding and quarantine
1640 measures. Moreover, a highly aggressive pathotype might repress the host's defense mechanisms,
1641 creating favorable conditions for the less virulent pathotypes. Thus, an even more diverse
1642 population of sunflower downy mildew can threaten the effectiveness of control methods against
1643 the pathogen. Integrated pest management, therefore, is an essential tool to manage *Plasmopara*
1644 *halstedii*. In addition, the introduction of new methods in pathotyping is urgent because of the
1645 uncertainties of previous methods.

1646 The widespread use of mefenoxam has resulted in a decline in efficacy against sunflower downy
1647 mildew in Hungary, in Europe and in the USA. Mefenoxam performed poorly or only moderately
1648 in the case of several *P. halstedii* isolates in our study. In conclusion, treatment with mefenoxam
1649 had different effects on different *P. halstedii* isolates, according to disease rates and plant heights.
1650 Further studies are needed to explore the reasons for the differences in tissue responses to sensitive
1651 and tolerant isolates of *P. halstedii* in sunflower. In addition, how plant defense mechanism
1652 contributes to the effectiveness of fungicides also has to be elucidated.

1653 Fungicidal resistance/tolerance to mefenoxam requires the introduction of newer, effective agents
1654 to protect against the pathogen. This also calls for the research and introduction of new alternative
1655 control methods and innovative management tools against the disease. For this, the effect of two
1656 different neem-derived pesticides, such as neem leaf extract (NLE) and NeemAzal T/S, was tested
1657 in different concentrations against *P. halstedii* isolates under *in vitro* and *in vivo* in our study.

1658 Host tissue responses of neem-treated sunflowers inoculated by *P. halstedii* were examined for the
1659 first time by fluorescent microscope in our study. This research on neem-derived pesticides
1660 efficacy against downy mildew is a first step to control this disease. Further research is needed for
1661 alternative methods. Botanical pesticides, such as neem products may play an important and
1662 effective method in the future against *P. halstedii* and other pathogens.

7. NEW SCIENTIFIC RESULTS

I. We proved the dominance of high virulent pathotypes such as 704 and the presence of less virulent pathotypes (700 and 730) between 2017 and 2019 in Hungary. We first showed a highly virulent pathotype (724) in the western part of Hungary.

II. We identified pathotype 734 for the first time in Hungary and Central Europe and prooved its occurrence from three different regions of Hungary.

III. We confirmed the previous statements that there is a shift in Hungary's pathotype composition of sunflower downy mildew towards highly virulent pathotypes.

IV. We proved the presence of mefenoxam tolerant/resistant *P. halstedii* isolates in the Hungarian sunflower downy mildew population.

V. We first revealed differences in host tissue responses such as hypersensitive reaction and cell necrosis of mefenoxam-treated susceptible sunflowers inoculated with various *P. halstedii* isolates.

VI. We first found that neem leaf extract and azadirachtin were effective against two *P. halstedii* isolates *in vivo* and *in vitro.*

VII. We first observed a similar host tissue response (cell necrosis) in neem-treated and inoculated plants as previously observed in BTH (benzothiadiazole as Bion 50WG) treatments against sunflower downy mildew.

8. SUMMARY

Downy mildew of sunflower (*Helianthus annuus* L.), caused by the obligate biotrophic oomycete *Plasmopara halstedii* Farl. Berl. et de Toni, is one of the most destructive pathogens of sunflowers worldwide. In the absence of resistant sunflower cultivars and seed treatment, it can cause complete loss or decline in yield production. *Plasmopara halstedii* rapidly develops pathotypes that can break down the resistance genes in sunflowers. Therefore, knowing the virulence diversity within the pathogen population of sunflower downy mildew has become essential for resistance breeding and quarantine measures. Fungicide resistance of the pathogen is another increasing problem worldwide.

In view of the above, I have set the following objectives for my work: (i) Pathotype identification of *P. halstedii* (sunflower downy mildew) isolates collected from different regions in Hungary in three consecutive years (2017-2019), (ii) Testing the mefenoxam sensitivity of *P. halstedii* isolates collected in Hungary and characterize host tissue responses to tolerant/resistant isolates with fluorescence microscope, (iii) Investigations on the effectiveness of neem-derived pesticides on *P. halstedii* in sunflower under *in-vitro* and *in-vivo* conditions

As a result of our survey, pathotype 704 was the most widespread in the collected samples of *P. halstedii*. The presence of less virulent pathotypes (700 and 730) was also confirmed in our study from 2017 to 2019. During the study, 734 was identified as a new pathotype in Hungary. We confirmed the previous statements that there is a shift in Hungary's pathotype composition of sunflower downy mildew towards highly virulent pathotypes.

Mefenoxam performed poorly or only moderately in the case of several *P. halstedii* isolates in our test. Microscopic studies allowed us to estimate the sensitivity (tolerance) more accurately. We first revealed a clear difference in host tissue responses of mefenoxam-treated susceptible sunflowers inoculated with various *P. halstedii* isolates. The effects of different mefenoxam concentrations were also tested against 5 isolates of *P. halstedii*. From these, mefenoxam was only found to be effective at several folds of the registered concentration (3 mg/kg seeds) against one isolate (Mád).

Both concentrations of neem leaf extracts and NeemAzal T/S significantly reduced the sporulation and chlorosis of *P. halstedii* isolates as compared to inoculated control plants. We first observed a similar host tissue response (cell necrosis) in neem-treated and inoculated plants as previously observed in BTH (benzothiadiazole as Bion 50WG) treatments against sunflower downy mildew.

9. ACKNOWLEDGEMENTS

During the preparation of my PhD thesis, I had to take the help and guidance of some respected persons, who deserves my deepest gratitude. Therefore, I would like to thank a number of people for their help, support and invaluable contributions.

Firstly, I would like to express my sincere gratitude to Prof. Dr. József Kiss for giving me opportunity to make this journey start. I am indebted to him for his continued guidance and endless support all these years to me. Most importantly, I would like to show gratitude to my supervisor, Associate Professor Dr. Rita Bán for her marvelous supervision, support and encouragement. Her patience, enthusiasm, co-operations and suggestions made me present this research work to produce in the present form. I see her as the kindest pillar of strength, motivation and knowledge who has never ceased to motivate me. I am thankful for the extraordinary experiences she arranged for me and for providing opportunities to grow professionally.

I would like to extend my genuine thanks to Dr. Katalin Körösi, Attila Kovács, Dr. Vinogradov Sergey, Éva Várallyai.

I am fortunate to have been a part of the Department of Integrated Plant Protection. A special thanks to all my team mates, Dr. Pratik Pravin Doshi, Ahmed Ibrahim Alrashid Yousif, Kevein Ruas, Arbnora Berisha, István Bóta, Bertold Sánta, Máté Lengyel for their constant help and support.

I would like to thank Andrea Nagy, Rita Baraksó, Erzsébet Várszegi Szörényiné, Tündér Ilona Szőcs, for their constant administrative, technical help and advices.

I want to take this opportunity to express my gratitude to Zsuzsanna Tassy, Csilla Kánai, Edit Szabadszállási from the International Relations Centre and Mónika Törökné Hajdú, Beáta Éva Kárpáti, Edit Simáné Dolányi from the PhD office for their patience, guidance and constant support with the administrative work.

I am also acknowledging to them, to whom I forgot to mention their name.

Additionally, this endeavour would not have been possible without the generous support from the Tempus Public Foundation for accepting me for the Stipendium Hungaricum scholarship, who financed my research.

Last, but not least, my family deserves endless gratitude; especially my father Mr. ChetRam Tripathi and mother Mrs. Beena Tripathi. Their constant love, support and belief in me has kept

my spirits and motivation high throughout my PhD. I would like to thank all my friends for constantly listening to me and for cracking jokes when things became too serious. I would also like to thank my nieces (Anshika and Nitya) for all the entertainment.

I would like to thank God, for letting me through all the difficulties. I have experienced your guidance day by day. You are the one who let me finish my degree. I will keep on trusting you for my future.

1773 **10. REFERENCES**

1774

1775 ACHIMU, P., SCHLÖSSER, E. (1992): Effect of neem seed extracts (*Azadirachta indica* A. Juss)
1776 against downy mildew (*Plasmopara viticola*) of grapevine. In *Practice Oriented Results on Use*
1777 *and Production of Neem-Lngredients. Proceedings of First Workshop*, 99-107. Giessen: Trifolio
1778 M GmbH.

1779 AHMED, S., DE LABROUHE, D. T., DELMOTTE, F. (2012): Emerging virulence arising from
1780 hybridisation facilitated by multiple introductions of the sunflower downy mildew pathogen
1781 *Plasmopara halstedii*. *Fungal Genetics and Biology*, 49 (10) 847-855. p.

1782 ALBOURIE, J. M., TOURVIEILLE, J., TOURVIEILLE DE LABROUHE, D. (1998): Resistance
1783 to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. *European Journal of*
1784 *Plant Pathology*, 104 (3) 235-242. p.

1785 ALLARD, C. (1978): Mildiou du tournesol (*Plasmopara halstedii* (Farl.) Berl. et de
1786 Toni). *Recherche concernant les modalités de l'infection naturelle. Inf Tech CETIOM*, 62 3-10. p.

1787 ANONYMOUS, (2015): Annu. Rep. of Sunflower, Indian Institute of Oilseed Research,
1788 Hyderabad 14-15.

1789 BACHLAVA, E., RADWAN, O. E., ABRATTI, G., TANG, S., GAO, W., HEESACKER, A. F.,
1790 BAZZALO, M. E., ZAMBELLI, A., LEON, A. J., KNAPP, S. J. (2011): Downy mildew (*Pl 8* and
1791 *Pl 14*) and rust (*R Adv*) resistance genes reside in close proximity to tandemly duplicated clusters
1792 of non-TIR-like NBS-LRR-encoding genes on sunflower chromosomes 1 and 13. *Theoretical and*
1793 *applied genetics*, 122 (6) 1211-1221. p.

1794 BALL, B. C., BINGHAM, I., REES, R. M., WATSON, C. A., LITTERICK, A. (2005): The role
1795 of crop rotations in determining soil structure and crop growth conditions. *Canadian Journal of*
1796 *Soil Science*, 85 (5) 557-577. p.

1797 BÁN, R., BAGLYAS, G., VIRÁNYI, F., BARNA, B., POSTA, K., KISS, J., KÖRÖSI, K. (2017):
1798 The chemical inducer, BTH (benzothiadiazole) and root colonization by mycorrhizal fungi
1799 (Glomus spp.) trigger resistance against white rot (*Sclerotinia Sclerotiorum*) in sunflower. *Acta*
1800 *Biologica Hungarica,* 68 (1) 50-59. p.

1801 BÁN, R., KOVÁCS, A., BAGLYAS, G., PERCZEL, M., ÉGEI, M., TURÓCZI, G., KÖRÖSI, K.
1802 (2016): Napraforgó-peronoszpóra (*Plasmopara halstedii* (Farl.) Berl. et de Toni) patotípusok
1803 elterjedése hazánkban. *Növényvédelem,* 52 391-396. p.

1804 BÁN, R., KOVÁCS, A., KÖRÖSI, K., PERCZEL, M., TURÓCZI, G. (2014): First report on the
1805 occurrence of a new pathotype, 714, of *Plasmopara halstedii* (sunflower downy mildew) in
1806 Hungary. *Plant Disease,* 98 (11) 1580-1580. p.

1807 BÁN, R., KOVÁCS, A., KÖRÖSI, K., PERCZEL, M., TURÓCZI, G., ZALAI, M., PÁLINKÁS,
1808 Z., ÉGEI, M. (2018): First report on the occurrence of a globally new pathotype, 724, of
1809 *Plasmopara halstedii* causing sunflower downy mildew in Hungary. *Plant disease*, 102 (9) 1861.
1810 p.

1811 BÁN, R., KOVÁCS, A., NISHA, N., PÁLINKÁS, Z., ZALAI, M., YOUSIF, A. I. A., KÖRÖSI,
1812 K. (2021): New and High Virulent Pathotypes of Sunflower Downy Mildew (*Plasmopara*
1813 *halstedii*) in Seven Countries in Europe. *Journal of Fungi,* 7 (7) 549. p.

1814 BÁN, R., VIRÁNYI, F., KOMJÁTI, H. (2004): Benzothiadiazole-induced resistance to
1815 *Plasmopara halstedii* (Farl.) Berl. et de Toni in sunflower. In Advances in Downy Mildew
1816 Research—Volume 2 (265-273). Springer, Dordrecht.

1817 BÁN, R., VIRÁNYI, F., NAGY, S., KÖRÖSI, K. (2004): Investigations of the induced resistance
1818 to *Plasmopara halstedii*. *Disease Resistance and Pathology.* Proc. 16th International Sunflower
1819 Conference, Fargo, ND USA, 89-91. p.

1820 BARILLI E. P., RATS E., RUBIALES, D. (2010): Benzothiadiazole and BABA improve
1821 resistance to *Uromyces pisi* (Pers.) Wint. In *Pisum sativum* L. with an enhancement of enzymatic
1822 activities and total phenolic content. *European Journal of Plant Pathology,* 128 483–493. p.

1823 BARZMAN, M., BÀRBERI, P., BIRCH, A. N. E., BOONEKAMP, P., DACHBRODT-
1824 SAAYDEH, S., GRAF, B., HOMMEL, B., JENSEN, J.E., KISS, J., KUDSK, P., LAMICHHANE,
1825 J. R. (2015): Eight principles of integrated pest management. *Agronomy for sustainable*
1826 *development,* 35 (4) 1199-1215. p.

1827 BERT, P. F., TOURVIEILLE DE LABROUHE, D., PHILIPPON, J., MOUZEYAR, S., JOUAN,
1828 I., NICOLAS, P., VEAR, F. (2001): Identification of a second linkage group carrying genes
1829 controlling resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*
1830 L.). *Theoretical and Applied Genetics*, 103 (6) 992-997. p.

1831 BHUVANESWARI, V., SRIVASTAVA, A. K., PAUL, P. K. (2012): Aqueous fruit extracts of
1832 *Azadirachta indica* induce systemic acquired resistance in barley against *Drechslera*
1833 *graminea*. *Archives of Phytopathology and Plant Protection*, 45 (8) 898-908. p.

1834 BISWAS, K., CHATTOPADHYAY, I., BANERJEE, R. K., BANDYOPADHYAY, U. (2002): 1835 Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science,* 82 1836 (11) 1336-1345. p.

1837 BRASIER, C. M. (1995): Episodic selection as a force in fungal microevolution, with special 1838 reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* 73 S1213– 1839 S1221. p.

1840 BRENT, K. J., HOLLOMON, D.W. (1998): *Fungicide resistance: the assessment of risk* (Vol. 2). 1841 Brussels, Belgium: Global Crop Protection Federation.

1842 BUZI, A., CHILOSI, G., DE SILLO, D., MAGRO, P. (2004): Induction of resistance in melon to 1843 *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with acibenzolar-S-methyl 1844 and methyl jasmonate but not with salicylic acid. *Journal of Phytopathology,* 152 (1) 34-42. p.

1845 CAHILL, D. M., MORRIS, P. F., WARD, E. W. B. (1993): Influence of metalaxyl on abscisic 1846 acid levels in soybean hypocotyls infected with *Phytophthora sojae*. *Physiological and molecular* 1847 *plant pathology*, 42 (2) 109-121. p.

1848 CAHILL, D. M., WARD, E. W. B. (1989): Effect of metalaxyl on elicitor activity, stimulation of 1849 glyceollin production and growth of sensitive and tolerant isolates of *Phytophthora* 1850 *megasperma* f.sp.*glycinea*. *Physiological and Molecular Plant Pathology*, 35 97-112. p.

1851 CASTAÑO, F. D. (2018): The sunflower crop in Argentina: Past, present and potential future. 1852 OCL: Oilseeds Fats Crops Lipids 25 D105. p.

1853 CHOI, Y. J., KISS, L., VAJNA, L., SHIN, H. D. (2009): Characterization of a Plasmopara species 1854 on *Ambrosia artemisiifolia*, and notes on *P. halstedii*, based on morphology and multiple gene 1855 phylogenies. *Mycological Research,* 113 1127-1136. p.

1856 COHEN, Y. R. (2002): β-aminobutyric acid-induced resistance against plant pathogens. *Plant* 1857 *disease*, 86 (5) 448-457. p.

1858 COHEN, Y., SACKSTON, W. E. (1973): Factors affecting infection of sunflowers by *Plasmopara* 1859 *halstedii*. *Canadian Journal of Botany*, 51 (1) 15-22. p.

1860 CONRATH, U. (2006): Systemic acquired resistance. *Plant signaling and behavior*, 1 (4) 179- 1861 184. p.

1862 CONRATH, U., PIETERSE, C. M., MAUCH-MANI, B. (2002): Priming in plant–pathogen 1863 interactions. *Trends in plant science*, 7 (5) 210-216. p.

1864 COVARELLI, L., TOSI, L. (2006): Presence of sunflower downy mildew in an integrated weed
1865 control field trial. *Journal of phytopathology*, 154 (5) 281-285. p.

1866 DAVIDSE, L. C. (1995): Phenylamide fungicides. Biochemical action and resistance. Pages
1867 347354 in: Modern Selective Fungicides: Properties, Applications, Mechanisms of Action. H. Lyr,
1868 ed. Gustav Fisher Verlag, New York.

1869 DAVIDSE, L. C., HOFMAN, A. E., VELTHUIS, G. C. M. (1983): Specific interference of
1870 metalaxyl with endogenous RNA polymerase activity in isolated nuclei from *Phytophthora*
1871 *megasperma* f. s *medicaginis*. *Experimental Mycolology,* 7 344-361.

1872 DE LABROUHE, D. T., GULYA, T. J., MASIREVIC, S., PENAUD, A., RASHID, K. Y.,
1873 VIRANYI, F. (2000, June): New nomenclature of races of *Plasmopara halstedii* (sunflower
1874 downy mildew). In *Proceedings of the 15th International Sunflower Conference* (pp. 61-66).

1875 DE ROMANO, A. B., ROMANO, C., BULOS, M., ALTIERI, E., SALA, C. (2010, June): A new
1876 gene for resistance to downy mildew in sunflower. In *Proceedings of International Symposium*
1877 *"Sunflower Breeding on Resistance to Diseases," Krasnodar, Russia* (pp. 141-146).

1878 DELMOTTE, F., GIRESSE, X., RICHARD-CERVERA, S., M'BAYA, J., VEAR, F.,
1879 TOURVIEILLE, J., WALSER, P., DE LABROUHE, D. T. (2008): Single nucleotide
1880 polymorphisms reveal multiple introductions into France of *Plasmopara halstedii*, the plant
1881 pathogen causing sunflower downy mildew. *Infection, Genetics and Evolution*, 8 (5) 534-540. p.

1882 DELOS, M., PENAUD, A., LAFON, S., WALSER, P., DE GUENIN, M. C. (1997): Le mildiou
1883 du tournesol: Une maladie toujours d'actualité. *Phytoma, la défense des végétaux*, (495) 15-16.

1884 DOSHI, P., NISHA, N., YOUSIF, A. I. A., KÖRÖSI, K., BÁN, R., TURÓCZI, G. (2020):
1885 Preliminary Investigation of Effect of Neem-Derived Pesticides on *Plasmopara halstedii*
1886 Pathotype 704 in Sunflower under *In Vitro* and *In Vivo* Conditions. *Plants*, 9 (4) 535. p.

1887 DOSHI, P., PÓSS, A. M., TÓTH, F., SZALAI, M., TURÓCZI, G. (2018): Effect of neem-derived
1888 plant protection products on the isopod species Porcellionidespruinosus (Brandt, 1833). *ZooKeys*,
1889 (801) 415-425. p.

1890 Durrant, W. E., Dong, X. (2004): Systemic acquired resistance. *Annual review of phytopathology,*
1891 42 (1) 185-209. p.

1892 ENJALBERT, J., DUAN, X., LECONTE, M., HOVMØLLER, M. S., DE VALLAVIEILLE-
1893 POPE, C. (2005): Genetic evidence of local adaptation of wheat yellow rust (*Puccinia striiformis*
1894 f. sp. *tritici*) within France. *Molecular ecology*, 14 (7) 2065-2073. p.

1895 ER, Y., ÖZER, N., KATIRCIOĞLU, Y. Z. (2021): In vivo anti-mildew activity of essential oils
1896 against downy mildew of sunflower caused by *Plasmopara halstedii*. *European Journal of Plant*
1897 *Pathology*, *161* (3) 619-627. p.

1898 FARLOW, W. G. (1882), May: Notes on some species in the third and eleventh centuries of Ellis's
1899 North American Fungi. In *Proceedings of the American Academy of Arts and Sciences*,18 65-85.
1900 *American Academy of Arts & Sciences*.

1901 FAWKE, S., DOUMANE, M., SCHORNACK, S. (2015): Oomycete interactions with plants:
1902 infection strategies and resistance principles. *Microbiology and Molecular Biology Reviews*, 79
1903 (3) 263-280. p.

1904 FERNANDEZ, A. M., GÓMEZ-RODRÍGUEZ, M.V., VELASCO-NEGUERUELA, A.,
1905 CAMACHO-SIMARRO, A.M., FERNÁNDEZ-LÓPEZ, C., ALTAREJOS, J. (2004): I*n Vivo*
1906 Antifungal Activity of the Essential Oil of *Bupleurum gibraltarium* against *Plasmopara halstedii*
1907 in Sunflower. *Journal of Agriculture and Food Chemistry,* 52 6414. p.

1908 FICK, G. N., ZIMMER, D. E., (1974): RHA271, RHA273 and RHA274 sunflower parental lines
1909 for producing downy mildew resistant hybrids. *Farm Research;* 32 2; *Nov/Dec 1974*.

1910 FISHER, D. J., AND HAYES, A. L. (1984): Studies of mechanisms of metalaxyl fungitoxicity
1911 and resistance to metalaxyl. *Crop Protection*, 3 (2) 177-185. p.

1912 FRIEDRICH, L., LAWTON, K., RUESS, W., MASNER, P., SPECKER, N., RELLA, M.G.,
1913 MEIER, B., DINCHER, S., STAUB, T., UKNES, S., MÉTRAUX, J. P. (1996): A
1914 benzothiadiazole derivative induces systemic acquired resistance in tobacco. *The Plant*
1915 *Journal*, 10 (1) 61-70. p.

1916 FRISKOP, A., MARKELL, S. G., GULYA, T. (2009): Downy mildew of sunflower.

1917 GARCÍA-CARNEROS, A. B., MOLINERO-RUIZ, L. (2017): First report of the highly virulent
1918 race 705 of *Plasmopara halstedii* (downy mildew of sunflower) in Portugal and in Spain. *Plant*
1919 *Disease*, 101 (8) 1555-1555. p.

1920 GASCUEL, Q., BUENDIA, L., PECRIX, Y., BLANCHET, N., MUÑOS, S., VEAR, F.,
1921 GODIARD, L. (2016): RXLR and CRN effectors from the sunflower downy mildew pathogen
1922 *Plasmopara halstedii* induce hypersensitive-like responses in resistant sunflower lines. *Frontiers*
1923 *in Plant Science*, 7 1887. p.

1924 GASCUEL, Q., MARTINEZ, Y., BONIFACE, M. C., VEAR, F., PICHON, M., AND GODIARD,
1925 L. (2015): The sunflower downy mildew pathogen *Plasmopara halstedii*. *Molecular Plant*
1926 *Pathology,* 16 (2, 109-122. p.

1927 GHAZANFAR, M. U., WAKIL, W., SAHI, S. T. (2011): Induction of resistance in chickpea
1928 (*Cicer arietinum* L.) against *Ascochyta rabiei* by applying chemicals and plant extracts. *Chilean*
1929 *Journal of Agricultural Research*, 71 (1) 52. p.

1930 GILLEY, M. A., GULYA, T. J., SEILER G. J., UNDERWOOD, W., HULKE, B. S., MISAR, C.
1931 G., MARKELL, S.G. (2020): Determination of virulence phenotypes of *Plasmopara halstedii*
1932 in the United States. *Plant Disease,* 104 2823-2831. p.

1933 GIRISH, K., BHAT, S. S. (2008): Neem – A Green Treasure. *Electronic Journal of Biology,* 4 (3)
1934 102-111. p.

1935 GOOSSEN, P. G., SACKSTON, W. E. (1968).: Transmission and biology of sunflower downy
1936 mildew. *Canadian Journal of Botany*, 46 (1) 5-10. p.

1937 GÖRE, M. E. (2009): Epidemic outbreaks of downy mildew caused by *Plasmopara halstedii* on
1938 sunflower in Thrace, part of the Marmara region of Turkey. *Plant Pathology*, 58 396. p.

1939 GÖRLACH, J., VOLRATH, S., KNAUF-BEITER, G., HENGY, G., BECKHOVE, U., KOGEL,
1940 K.H., OOSTENDORP, M., STAUB, T., WARD, E., KESSMANN, H., RYALS, J. (1996):
1941 Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene
1942 expression and disease resistance in wheat. *The Plant Cell*, 8 (4) 629-643. p.

1943 GRAY, A. B., SACKSTON, W. E. (1985): Early stages of infection of resistant and susceptible
1944 sunflower seedlings by three races of *Plasmopara halstedii*. *Canadian journal of botany*, 63 (10)
1945 1725-1729. p.

1946 GULYA, T. J. (2000), June. Metalaxyl resistance in sunflower downy mildew and control through
1947 genetics and alternative fungicides. In *Proceedings of the 15th International Sunflower*
1948 *Conference, Toulouse, France* (Vol. 2).

1949 GULYA, T. J. (2007).: Distribution of *Plasmopara halstedii* races from sunflower around the
1950 world. *Advances in downy mildew research*, 3 121-134. p.

1951 GULYA, T. J., MARKELL, S., MCMULLEN, M., HARVESON, B., OSBORNE, L. (2011): New
1952 virulent races of downy mildew: distribution, status of DM resistant hybrids, and USDA sources
1953 of resistance. In Proceedings of 33th Sunflower Research Forum. National Sunflower Association,
1954 Fargo ND, January, 12-13.

1955 GULYA, T. J., TOURVIEILLE DE LABROUHE, D., MASIREVIC, S., PENAUD, A., RASHID,
1956 K., VIRANYI, F. (1998): Proposal for the standardised nomenclature and identification of races
1957 of *Plasmopara halstedii* (sunflower downy mildew). In: Sunflower Downy Mildew Symposium,
1958 Proceedings of Sunflower Downy Mildew Symposium, International Sunflower Association
1959 Symposium III, Fargo, ND, USA, pp. 130– 136. dii Causing Sunflower Downy Mildew in
1960 Hungary. *Plant disease*, 102 (9) 1861. p.

1961 GULYA, T., KANDEL, H., MCMULLEN, M., KNODEL, J., BERGLUND, D., MATHEW, F.,
1962 LAMEY, H. A., NOWATZKI, J., MARKELL, S. (2013): Prevalence and incidence of sunflower
1963 downy mildew in North Dakota between 2001 and 2011. *Plant Health Progress,* 14 (1) 20. p.

1964 GULYA, T., RASHID, K. Y., MASIREVIC, S. M. (1997): Sunflower diseases. *Sunflower*
1965 *technology and production*, *35*, pp.263-379. p.ASA Series of Monographs, vol. 35. The American
1966 Society of Agronomy, Madison, USA, 263–379.

1967 HAHN, M. (2014): The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as
1968 a case study. *Journal of chemical biology,* 7 (4) 133-141. p.

1969 HASAN, M. M., CHOWDHURY, S. P., ALAM, S., HOSSAIN, B., ALAM, M.S., 2005.
1970 Antifungal Effects of Plant Extracts on Seed-borne Fungi of Wheat Seed Regarding Seed
1971 Germination, Seedling Health and Vigour Index. *Pakistan Journal of Biological Sciences,* 8 1284-
1972 1289. p.

1973 HAYAT, Q., HAYAT, S., IRFAN, M., AHMAD, A. (2010): Effect of exogenous salicylic acid
1974 under changing environment: a review. *Environmental and Experimental Botany,* 68 (1) 14-25. p.

1975 HEIL, M., BOSTOCK, R. M. (2002): Induced systemic resistance (ISR) against pathogens in the
1976 context of induced plant defences. *Annals of botany*, 89 (5) 503-512. p.

1977 HELLER, A., ROZYNEK, B., SPRING, O., (1997): Cytological and physiological reasons for the
1978 latent type of infection in sunflower caused by *Plasmopara halstedii*. *Journal of*
1979 *Phytopathology*, 145 (10) 441-445. p.

1980 HERMANNS, M., SLUSARENKO, A. J., SCHLAICH, N. L. (2003): Organ specificity in a plant
1981 disease is determined independently of R gene signalling. *Molecular Plant–Microbe Interactions,*
1982 16 752–759. p.

1983 HICKEY, E. L., COFFEY, M. D. (1980): The effects of Ridomil on *Peronospora pisi* parasitizing
1984 *Pisum sativum*: an ultrastructural investigation. *Physiological Plant Pathology*, 17 (2) 199-204. p.

1985 HUMANN, R. M., JOHNSON, K. D., WUNSCH, M. J., MEYER, S. M., JORDAHL, J. G.,
1986 BAUSKE, E. C., HALVORSON, J. M., FRISKOP, A. J., O'BRYAN, K. A., GULYA, T. J.,
1987 MARKELL, S. G. (2019): Evaluation of oxathiapiprolin for the management of sunflower downy
1988 mildew. *Plant Disease*, 103 (10) 2498-2504.

1989 HUMANN, R., GULYA, T. J., MARKELL, S. G. (2016): Downy mildew. Pages 18-20in:
1990 Compendium of Sunflower Diseases. R. M. Harveson, S. G. Markell, C. C. Block, and T. J. Gulya,
1991 eds. *American Phytopathological Society,* St. Paul, MN, U.S.A

1992 IOOS, R., LAUGUSTIN, L., ROSE, S., TOURVIEILLE, J., AND TOURVIEILLE DE
1993 LABROUHE, D. (2007): Development of a PCR test to detect the downy mildew causal agent
1994 *Plasmopara halstedii* in sunflower seeds. *Plant Pathology*, 56 (2) 209-218. p.

1995 IWEBOR M., ANTONOVA T. S., SAUKOVA S. (2016): Changes in the racial structure of
1996 *Plasmopara halstedii* (Farl.) Berl. et de Toni population in the South of the Russian Federation.
1997 *Helia,* 39 113-121. p.

1998 IWEBOR M., ANTONOVA T., SAUKOVA S., ARASLANOVA N. (2019): Sunflower downy
1999 mildew in the south of Russia. *Zashchita i Karantin Rasteniy,* 10 29-33. Russian

2000 IWEBOR, M., ANTONOVA, T., ARASLANOVA, N., SAUKOVA, S., PITINOVA, Y.,
2001 ELISEEVA, K., BELORUTSKIY, A. (2021): The first report of *Plasmopara halstedii* race 337 in
2002 the Russian Federation. *Plant Protection Science*, 58 (1) 76-80. p.

2003 IWEBOR, M., ANTONOVA, T., SAUKOVA, S. (2018): Occurrence and distribution of races
2004 713, 733 and 734 of sunflower downy mildew pathogen in the Russian Federation. *Helia*, 41 (69)
2005 141-151. p.

2006 JAKAB G., COTTIER V., TOQUIN V., RIGOLI G., ZIMMERLI L., METRAUX J., MAUCH-
2007 MANI B. (2001): β-aminobutyric acid-induced resistance in plants. *European Journal of Plant*
2008 *Pathology* 107 29–37. p.

2009 JAYARAJ, J., MUTHUKRISHNAN, S., LIANG, G. H., VELAZHAHAN, R. (2004): Jasmonic
2010 acid and salicylic acid induce accumulation of β-1, 3-glucanase and thaumatin-like proteins in
2011 wheat and enhance resistance against Stagonospora nodorum. *Biologia Plantarum*, 48 (3) 425-
2012 430. p.

2013 JI, P., CSINOS, A. S., HICKMAN, L. L., HARGETT, U. (2014): Efficacy and application
2014 methods of oxathiapiprolin for management of black shank on tobacco. *Plant Disease,* 98 (11)
2015 1551-1554. p.

2016 JOCIĆ, S., MILADINOVIĆ, D., IMEROVSKI, I., DIMITRIJEVIĆ, A., CVEJIĆ, S., NAGL, N.,
2017 KONDIĆ-ŠPIKA, A. (2012): Towards sustainable downy mildew resistance in sunflower. *Helia,*
2018 35 (56) 61-72. p.

2019 JUSTYNA, P.G., EWA, K., (2013): Induction of resistance against pathogens by β-aminobutyric
2020 acid. *Acta Physiologiae Plantarum*, 35 (6) 1735-1748. p.

2021 KALTZ, O., SHYKOFF, J. A. (1998): Local adaptation in host-parasite systems. *Heredity,* 81
2022 361–370. p.

2023 KAYA, Y., JOCIĆ, S., Miladinovic, D. (2012): Sunflower. In S. K. Gupta (Ed.) Technological
2024 Innovations in Major World Oil Crops, Vol. 1. 85-130. p.

2025 KEMA, G. H. J., GOHARI, A. M., AOUINI, L., GIBRIEL, H. A. Y., WARE, S. B. et al (2018):
2026 Stress and sexual reproduction affect the dynamics of the wheat pathogen effector *AvrStb6* and
2027 strobilurin resistance. *Nature Genetics,* 50 375–380. p.

2028 KEPCZYNSKA, E., KEPCZYNSKI, J. (2005): Inhibitory effect of methyl jasmonate on
2029 development of phytopathogen *Aletrnaria alternata* (Fr.) Keissl and its reversal by ethephon and
2030 ACC. Acta Physiologiae Plantarum, 27 491-496. p.

2031 KĘPCZYŃSKA, E., KRÓL, P. (2011): The phytohormone methyl jasmonate as an activator of
2032 induced resistance against the necrotroph *Alternaria porri* f. sp. solani in tomato plants. *Journal*
2033 *of Plant Interactions,* 7 (4) 307-315. p.

2034 KINMAN, M. L. (1970): New developments in the USDA and state experiment station sunflower
2035 breeding programs. In: *Proc. 4th Int. Sunflower Conf.* Memphis, TN, USA, 181-183.

2036 KNESS, A. A., JOHNSON, G., EVERTS, K. L., EVANS, T. A., DONOFRIO, N. M., ERNEST,
2037 E. G. (2016): Managing pod rot of lima bean caused by *Phytophthora capsica* with fungicides in
2038 the Mid-Atlantic region. *Plant Health Progress,* 17 (2) 130-132. p.

2039 KÖRÖSI, K., BÁN, R., BARNA, B., VIRÁNYI, F. (2011): Biochemical and molecular changes
2040 in downy mildew-infected sunflower triggered by resistance inducers. *Journal of Phytopathology*,
2041 159 (7-8) 471-478. p.

2042 KÖRÖSI, K., KOVÁCS, A., NISHA, N., BÓTA, I., PERCZEL, M., YOUSIF, A. I. A., KISS, J.,
2043 BÁN, R. (2020): New data on pathotype distribution and mefenoxam tolerance of *Plasmopara*
2044 *halstedii* in Hungary. *Plant Protection Science*, 57 (1) 31-37. p.

2045 KÖRÖSI, K., LÁZÁR, N., VIRÁNYI, F. (2009): Resistance to downy mildew in sunflower
2046 induced by chemical activators. *Acta phytopathologica et entomologica Hungarica*, 44 (1) 1-9. p.

2047 KRZYZANIAK, Y., TROUVELOT, S., NEGREL, J., ET A. L. (2018): A plant extract acts both
2048 as a resistance inducer and an oomycide against grapevine downy mildew. *Frontiers in Plant*
2049 *Science,* 1085, 9. p.

2050 KUĆ, J. (2001): Concepts and direction of induced systemic resistance in plants and its
2051 application. *European Journal of Plant Pathology,* 107 (1) 7-12. p.

2052 LAFON, S., PENAUD, A., WALSER, P., DE GUENIN, M. C., MOLINERO, V., MESTRES, R.,
2053 TOURVIEILLE, D. (1996): Le mildiou du tournesol toujours sous surveillance. *Phytoma-La*
2054 *Défense des Végétaux,* 484 35-36. p.

2055 LAMOUR, K. H., HAUSBECK, M. K. (2000): Mefenoxam insensitivity and the sexual stage of
2056 *Phytophthora capsici* in Michigan cucurbit fields. *Phytopathology*, 90 (4) 396-400. p.

2057 LAZAROVITS, G., WARD, E. W. B. (1982): Relationship between localized glyceollin
2058 accumulation and metalaxyl treatment in the control of Phytophthora rot in soybean hypocotyls.
2059 *Phytopathology,* 72 1217-1221. p.

2060 LIU, Z., GULYA, T. J., SEILER, G. J., VICK, B. A., JAN, C. C. (2012): Molecular mapping of
2061 the *Pl 16* downy mildew resistance gene from HA-R4 to facilitate marker-assisted selection in
2062 sunflower. *Theoretical and Applied Genetics*, 125 (1) 121-131. p.

2063 MA, G. J., SONG, Q. J., MARKELL, S. G., QI, L. L. (2018): High-throughput genotyping-by-
2064 sequencing facilitates molecular tagging of a novel rust resistance gene, *R15*, in sunflower
2065 (*Helianthus annuus* L.). *Theoretical and Applied Genetics,* 131 (7) 1423-1432. p.

2066 MA, G., SONG, Q., UNDERWOOD, W. R., ZHANG Z. W., FIEDLER, J. D., XUEHUI, L., QI,
2067 L. (2019): Molecular dissection of resistance gene cluster and candidate gene identification of *Pl17*
2068 and *Pl19* in sunflower by whole-genome resequencing. *Scientific Reports,* 9 14974. p.

2069 MALECK, K., LEVINE. A., EULGEM, T. (2000): The transcriptome of *Arabidopsis thaliana*
2070 during systemic acquired resistance. *Nature Genetics,* 26 403-410. p.

2071 MARKELL, S. G., HARVESON, R. M., BLOCK, C. C., GULYA, T. J. (2015): Sunflower
2072 diseases. In: E. Martínez-Force, N.T. Dunford, and J.J. Salas, editors, Sunflower: Chemistry,
2073 production, processing, an d utilization. AOCS Press, Urbana, IL. 93–128.

2074 MARTÍN-SANZ, A., RUEDA, S., GARCÍA-CARNEROS, A. B., MOLINERO-RUIZ, L. (2020):
2075 First report of a new highly virulent pathotype of sunflower downy mildew (*Plasmopara halstedii*)
2076 overcoming the *Pl8* resistance gene in Europe. *Plant Disease,* 104 (2) 597. p.

MARTÍN-SANZ, A., RUEDA, S., GARCÍA-CARNEROS, A.B., MOLINERO-RUIZ, L. (2020): First Report of a New Highly Virulent Pathotype of Sunflower Downy Mildew (*Plasmopara halstedii*) Overcoming the *Pl8* Resistance Gene in Europe. *Plant Disease,* 104 597. p.

MCDONALD, B. A., LINDE, C. (2002): Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, 40, 439-479. p.

MELERO-VARA, J. M., GARCIA-BAUDIN, C., LOPEZ-HERRERA, C. J., JIMENEZ-DIAZ, R. M. (1982): Control of sunflower downy mildew with metalaxyl. *Plant Disease*, 66 (2) 132–135. p.

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 35-44. p.

MILLER, J. F., GULYA, T. J. (1984): Reaction of breeding lines and crosses to race 2 and race 3 of downy mildew. *In Proceedings of the Sunflower Research Workshop. National Sunflower Association,* Bismarck, ND. p. 10

MILLER, J. F., GULYA, T. J. (1991): Inheritance of resistance to race 4 of downy mildew derived from interspecific crosses in sunflower. *Crop science*, 31 (1) 40-43. p.

MIRANDA-FUENTES, P., GARCÍA-CARNEROS, A. B., MOLINERO-RUIZ, L. (2021): Updated characterization of races of *Plasmopara halstedii* and entomopathogenic fungi as endophytes of sunflower plants in axenic culture. *Agronomy,* 11 (2) 268. p.

MIRZA, J. I., HAMEED, S., AHMAD, I., AYUB, N., STRANG, R. (2000): *In Vitro* Antifungal Activity of Neem Products Against *Phytophthora Infestans*. *Pakistan Journal of Biological Sciences,* 3 (5) 824-828. p.

MIRZAHOSEIN-TABRIZI, M. (2017): Identification of downy mildew resistance loci in sunflower germplasm. *Notulae Scientia Biologicae*, 9 (4) 515-519. p.

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/reacción de las poblaciones campestres de tizón (*Plasmopara halstedii*) en girasol a metalaxil y mefenoxam/réactions des populations champêtres de rouille (*Plasmopara halstedii*) du tournesol au metalaxyl et au mefenoxam. *Helia,* 28 (42) 65-74. p.

2106 MOLINERO-RUIZ, M. L., MELERO-VARA, J. M., DOMÍNGUEZ, J. (2003a): Inheritance of
2107 resistance to two races of sunflower downy mildew (*Plasmopara halstedii*) in two *Helianthus*
2108 *annuus* L. lines. *Euphytica*, 131 (1) 47-51. p.

2109 MOLINERO-RUIZ, M. L., MELERO-VARA, J. M., GULYA, T. J., DOMINGUEZ, J. (2003b):
2110 First report of resistance to metalaxyl in downy mildew of sunflower caused by *Plasmopara*
2111 *halstedii* in Spain. *Plant Disease,* 87 (6) 749-749. p.

2112 MORTON, H. V., URECH, P. A. (1988): History of the development of resistance to phenylamide
2113 fungicides. Pages 59-60 in: Fungicide Resistance in North America. C. E. Delp, ed. *American*
2114 *Phytopathological Society Press*, St. Paul, MN.

2115 MOUZEYAR, S., DE LABROUHE, D. T., VEAR, F. (1993): Histopathological studies of
2116 resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii*). *Journal*
2117 *of Phytopathology*, 139 (4) 289-297. p.

2118 MOUZEYAR, S., DE LABROUHE, D. T., VEAR, F. (1994): Effect of Host-race Combination
2119 on Resistance of Sunflower, *Helianthus annuus* L., to Downy Mildew *Plasmopara halstedii*.
2120 *Journal of Phytopathology,* 141 (3) 249-258. p.

2121 MOUZEYAR, S., ROECKEL-DREVET, P., GENTZBITTEL, L., PHILIPPON, J.,
2122 TOURVIEILLE DE LABROUHE, D., VEAR, F., NICOLAS, P. (1995): RFLP and RAPD
2123 mapping of the sunflower *Pl1* locus for resistance to *Plasmopara halstedii* race 1. *Theoretical and*
2124 *Applied Genetics*, 91 (5) 733-737. p.

2125 MOUZEYAR, S., VEAR, F., DE LABROUHE, D. T. (1995): Microscopical studies of the effect
2126 of metalaxyl on the interaction between sunflower, *Helianthus annuus* L. and downy mildew,
2127 *Plasmopara halstedii*. *European journal of plant pathology*, 101 (4) 399-404. p.

2128 MOYERS, B. T., RIESEBERG, L. H. (2013): Divergence in gene expression is uncoupled from
2129 divergence in coding sequence in a secondarily woody sunflower. *International Journal of Plant*
2130 *Sciences*, 174 (7) 1079-1089. p.

2131 MULPURI, S., LIU, Z., FENG, J., GULYA, T. J., JAN, C. C. (2009): Inheritance and molecular
2132 mapping of a downy mildew resistance gene, Pl 13 in cultivated sunflower (*Helianthus annuus*
2133 L.). *Theoretical and Applied Genetics*, 119 (5) 795-803. p.

2134 NAGARAJU, A., MURALI, M., SUDISHA, J., AMRUTHESH, K. N., MURTHY, S. M. (2012a):
2135 Beneficial microbes promote plant growth and induce systemic resistance in sunflower against
2136 downy mildew disease caused by *Plasmopara halstedii*. *Current Botany*, 3 (5) 12-18. p.

2137 NAGARAJU, A., SUDISHA, J., MURTHY, S. M., ITO, S.I., (2012b): Seed priming with
2138 *Trichoderma harzianum* isolates enhances plant growth and induces resistance against
2139 *Plasmopara halstedii*, an incitant of sunflower downy mildew disease. *Australasian Plant*
2140 *Pathology*, 41 (6) 609-620. p.

2141 NANDEESHKUMAR, P., RAMACHANDRAKINI, K., PRAKASH, H. S., NIRANJANA, S. R.,
2142 SHEKAR SHETTY, H. (2008a): Induction of resistance against downy mildew on sunflower by
2143 rhizobacteria. *Journal of Plant Interactions,* 3 (4) 255-262. p.

2144 NANDEESHKUMAR, P., SAROSH, B. R., KINI, K. R., PRAKASH, H. S., SHETTY, H. S.
2145 (2009): Elicitation of resistance and defense related proteins by β-amino butyric acid in sunflower
2146 against downy mildew pathogen *Plasmopara halstedii*. *Archives of Phytopathology and Plant*
2147 *Protection,* 42 (11) 1020-1032. p.

2148 NANDEESHKUMAR, P., SUDISHA, J., RAMACHANDRA, K. K., PRAKASH, H. S.,
2149 NIRANJANA, S. R., SHEKAR, S. H. (2008b): Chitosan induced resistance to downy mildew in
2150 sunflower caused by *Plasmopara halstedii*. *Physiological and Molecular Plant Pathology,* 72 (4-
2151 6) 188-194. p.

2152 NISHA, N., KÖRÖSI, K., PERCZEL, M., YOUSIF, A. I. A., BÁN, R. (2021): First report on the
2153 occurrence of an aggressive pathotype, 734, of *Plasmopara halstedii* causing sunflower downy
2154 mildew in Hungary. *Plant Disease*, 105 (3) 711-711. p.

2155 NOVOTELNOVA, N.S., (1966): Downy mildew of sunflower. Izdatelstvo "Nauka", Moskva-
2156 Leningrad. Translated from Russian, Indian National Scientific Documentation, Hillside Road
2157 New Delhi.

2158 OOSTENDORP, M., KUNZ, W., DIETRICH, B., STAUB, T. (2001): Induced disease resistance
2159 in plants by chemicals. *European Journal of Plant Pathology*, 107 (1) 19-28. p.

2160 OROS, G., VIRÁNYI, F. (1984): Resistance of *Plasmopara halstedii* to metalaxyl in the
2161 greenhouse. *Temperate Downy Mildew Newsletter,* 3 22-23.

2162 PARRA, G., RISTAINO, J. B. (2001): Resistance to mefenoxam and metalaxyl among field
2163 isolates of *Phytophthora capsici* causing Phytophthora blight of bell pepper. *Plant Disease*, 85
2164 (10) 1069-1075. p.

2165 PARRA, G., RISTAINO, J. B., (2001): Resistance to mefenoxam and metalaxyl among field
2166 isolates of *Phytophthora capsici* causing *Phytophthora blight* of bell pepper. *Plant Disease,* 85
2167 (10) 1069-1075. p.

2168 PASTERIS, R. J., HANAGAN, M. A., BISAHA, J. J., FINKELSTEIN, B. L., HOFFMAN, L. E.,
2169 GREGORY, V., ANDREASSI, J. L., SWEIGARD, J. A., KLYASCHCHITSKY, B. A., HENRY,
2170 Y. T., BERGER, R. A. (2016): Discovery of oxathiapiprolin, a new oomycete fungicide that
2171 targets an oxysterol binding protein. *Bioorganic and Medicinal Chemistry,* 24 (3) 354-361. p.

2172 PATEL, J. S., COSTA DE NOVAES, M. I., ZHANG, S. (2015): Evaluation of the new compound
2173 oxathiapiprolin for control of downy mildew in basil. *Plant Health Progress,* 16 165-172.

2174 PATIL, M. A., PHAD, H., RAMTIRTHKAR, M. S., PHAD, H. B. (1991): Chemical control of
2175 sunflower downy mildew caused by *Plasmopara halstedii*. *Indian Phytopathology,* 44 325-327. p.

2176 PECRIX, Y., BUENDIA, L., PENOUILH-SUZETTE, C., MARECHAUX, M., LEGRAND, L.,
2177 BOUCHEZ, O., RENGEL, D., GOUZY, J., COTTRET, L., VEAR, F., GODIARD, L. (2019):
2178 Sunflower resistance to multiple downy mildew pathotypes revealed by recognition of conserved
2179 effectors of the oomycete *Plasmopara halstedii*. *The Plant Journal,* 97 (4) 730-748. p.

2180 PINTORE, I., GILARDI, G., GULLINO, M. L., GARIBALDI, A. (2016).: Detection of
2181 mefenoxam-resistant strains of *Peronospora belbahrii*, the causal agent of basil downy mildew,
2182 transmitted through infected seeds. *Phytoparasitica,* 44 (4) 563-569. p.

2183 QI, L. L., FOLEY, M. E., CAI, X. W., AND GULYA, T. J. (2016): Genetics and mapping of a
2184 novel downy mildew resistance gene, *Pl 18*, introgressed from wild *Helianthus argophyllus* into
2185 cultivated sunflower (*Helianthus annuus* L.). *Theoretical and Applied Genetics*, 129 (4) 741-752.
2186 p.

2187 QI, L. L., LONG, Y. M., JAN, C. C., MA, G. J., AND GULYA, T. J. (2015): *Pl 17* is a novel gene
2188 independent of known downy mildew resistance genes in the cultivated sunflower (*Helianthus
2189 annuus* L.). *Theoretical and Applied Genetics*, 128 (4) 757-767. p.

2190 QI, L. L., TALUKDER, Z. I., HULKE, B. S., FOLEY, M. E., (2017): Development and dissection
2191 of diagnostic SNP markers for the downy mildew resistance genes *Pl Arg* and *Pl 8* and maker-
2192 assisted gene pyramiding in sunflower (*Helianthus annuus* L.). *Molecular Genetics and
2193 Genomics*, 292(3) 551-563. p.

2194 RADWAN, O., BOUZIDI, M. F., MOUZEYAR, S. (2011): Molecular characterization of two
2195 types of resistance in sunflower to *Plasmopara halstedii*, the causal agent of downy
2196 mildew. *Phytopathology*, 101 (8) 970-979. p.

2197 RADWAN, O., MOUZEYAR, S., VENISSE, J. S., NICOLAS, P., BOUZIDI, M. F. (2005):
2198 Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a

2199 delayed hypersensitive response within the hypocotyls. *Journal of Experimental Botany*, 56 (420)
2200 2683-2693. p.

2201 RASHID, A., AHMAD, I., IRAM, S., MIRZA, J. I., RAUF, C. A. (2004): Efficacy of different
2202 Neem (*Azadirachta indica* A. Juss) products against various life stages of *Phytophthora infestans*
2203 (Mont) De Bary. *Pakistan Journal of Botany,* 36 (4) 881-886. p.

2204 RAUF, S. (2019): Breeding strategies for sunflower (*Helianthus annuus* L.) genetic improvement.
2205 In *Advances in plant breeding strategies: industrial and food crops* (637-673). Springer, Cham.

2206 REGNAULT, Y., TOURVIEILLE, D., AND LES POINTS TECHNIQUES DU CETIOM. (1991):
2207 Mildiou, attention aux nouvelles races. *Les points techniques du CETIOM (ed) Les maladies du*
2208 *tournesol*, 30-36.

2209 ROECKEL-DREVET, P., GAGNE, G., MOUZEYAR, S., GENTZBITTEL, L., PHILIPPON, J.,
2210 NICOLAS, P., TOURVIEILLE DE LABROUHE, D., VEAR, F. (1996): Colocation of downy
2211 mildew (*Plasmopara halstedii*) resistance genes in sunflower (*Helianthus annuus*
2212 L.). *Euphytica*, 91 (2) 225-228. p.

2213 ROHILLA, R., SINGH, U. S., SINGH, R.L. (2002): Mode of action of acibenzolar-S-methyl
2214 against sheath blight of rice, caused by *Rhizoctonia solani* Kuhn. *Pest Management Science:*
2215 *formerly Pesticide Science,* 58 63-69. p.

2216 ROZYNEK, B., SPRING, O. (2000): Pathotypes of sunflower downy mildew in southern parts of
2217 Germany. *HELIA-NOVI SAD*, 23, 27-34.

2218 SACKSTON, W. E. (1992): On a treadmill: breeding sunflowers for resistance to disease. *Annual*
2219 *review of phytopathology,* 30 (1) 529-551. p.

2220 Sakr, N. (2009): Variation in aggressiveness of *Plasmopara halstedii* (sunflower downy mildew).
2221 *Journal of Plant Diseases and Protection,* 116 (6) 247-251. p.

2222 SAKR, N. (2011a): Aggressiveness in *Plasmopara halstedii* (sunflower downy mildew). *The*
2223 *Plant Pathology Journal,* 27 (2) 110-115. p.

2224 SAKR, N. (2011b): Evolution of pathogenicity in *Plasmopara halstedii* (sunflower downy
2225 mildew). *Archives of Phytopathology and Plant Protection,* 44 (15) 1432-1437. p.

2226 SAKR, N. (2011c): Relationship between aggressiveness and zoosporangia viability in
2227 *Plasmopara halstedii* (sunflower downy mildew). *Archives of Phytopathology and Plant*
2228 *Protection,* 44 (16) 1585-1594. p.

2229 SAKR, N. (2012): Virulence cost in *Plasmopara halstedii* (sunflower downy mildew). *Archives*
2230 *of Phytopathology and Plant Protection,* 45 (6) 646-651. p.

2231 SAKR, N. (2013): Pathogenic, morphological and genetic diversity in *Plasmopara halstedii*, the
2232 causal agent of sunflower downy mildew. *Acta Scientiarum. Agronomy*, 35 (1) 9-19. p.

2233 SAKR, N., DUCHER, M., TOURVIEILLE, J., WALSER, P., VEAR, F., DE LABROUHE, D. T.
2234 (2009): A method to measure aggressiveness of *Plasmopara halstedii* (Sunflower Downy
2235 Mildew). *Journal of Phytopathology*, 157 (2) 133-136. p.

2236 SAKR, N., DUCHER, M., TOURVIEILLE, J., WALSER, P., VEAR, F., DE LABROUHE, D. T.
2237 (2008): Variation in form and size of *Plasmopara halstedii* (sunflower downy mildew)
2238 zoosporangia. *Mycological progress*, 7 (4) 257-265. p.

2239 SAKR, N., TOURVIEILLE DE LABROUHE, D., WALSER, P., DUCHER, M., DELMOTTE,
2240 F., TOURVIEILLE, J., VEAR, F. (2011): Analyzing the influence of qualitative resistance
2241 selection pressure on variation of aggressiveness in *Plasmopara halstedii*. *Journal of Plant*
2242 *Protection Research,* 51 (2) 140-144. p.

2243 ŠAŠEK, V., NOVÁKOVÁ, M., DOBREV, P.I., VALENTOVÁ, O., BURKETOVÁ L. (2012): β-
2244 aminobutyric acid protects *Brassica napus* plants from infection by *Leptosphaeria maculans*:
2245 resistance induction or a direct antifungal effect? *European Journal of Plant Pathology,* 133 279–
2246 289. p.

2247 SCHMUTTERER, H. (1988): Potential of azadirachtin-containing pesticides for integrated pest
2248 control in developing and industrialized countries. *Journal of Insect Physiology*, 34 (7) 713-719.
2249 p.

2250 SCHRÖTER, J. (1886): Fam. Peronosporacei. In F. Cohn (Ed.), Kryptogamen-Flora von Schlesien
2251 (228–252). Breslau: J. U. Kern.

2252 SCHWINN, F. J., MARGOT, P. (1991): Control with chemicals. In: Ingram D.S., Williams P.H.
2253 (eds): *Phytophthora infestans*, the Cause of Late Blight of Potato. *Advances in Plant Pathology.*
2254 London, Academic Press, 225–265. p.

2255 SCHWINN, F. J., STAUB, T. (1987): Phenylamides and other fungicides against Oomycetes. In:
2256 Lyr H. (ed.): Modern Selective Fungicides: Properties, Applications, Mechanisms of Action. New
2257 York, Jena-Fischer-Verlag, 323–346.

2258 SEDLÁŘOVÁ, M., POSPÍCHALOVÁ, R., TROJANOVÁ, Z. D., BARTŮŠEK, T.,
2259 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 (3) 182-187. p.

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. p.

SEILER, G. J. (1991): Registration of 13 downy mildew tolerant interspecific sunflower germplasm lines derived from wild annual species. *Crop Science*, 31 1714-1716. p.

SEILER, G. J., JAN, C. C. (2014): Wild sunflower species as a genetic resource for resistance to sunflower broomrape (*Orobanche cumana* Wallr.). *Helia*, 37 (61) 129-139. p.

SERRANO, R. A., LUNA DEL CASTILLO, J., JORRÍN NOVO, J., FERNÁNDEZ OCAÑA, A., RODRIGUEZ, G. (2007): Chitinase and peroxidase activities in sunflower hypocotyls: effects of BTH and inoculation with *Plasmopara halstedii*. *Biologia plantarum*, 51 (1) 149-152. p.

SEYBOLD, H., DEMETROWITSCH, T. J., HASSANI, M. A., SZYMCZAK, S., REIM, E., HAUEISEN, J., LÜBBERS, L., RÜHLEMANN, M., FRANKE, A., SCHWARZ, K., STUKENBROCK, E. H. (2020): A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. *Nature Communications,* 11 1910. p.

SHARMA, K., BRUNS, C., BUTZ, A. F., FINCKH, M. R. (2012): Effects of fertilizers and plant strengtheners on the susceptibility of tomatoes to single and mixed isolates of *Phytophthora infestans*. *European Journal of Plant Pathology*, 133 (3) 739-751. p.

SHARMA, R., XIA, X., CANO, L. M., EVANGELISTI, E., KEMEN, E., JUDELSON, H., OOME, S., SAMBLES, C., VAN DEN HOOGEN, D. J., KITNER, M., KLEIN, J. (2015): Genome analyses of the sunflower pathogen *Plasmopara halstedii* provide insights into effector evolution in downy mildews and Phytophthora. *BMC genomics*, 16 (1) 1-23. p.

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. p.

SILLERO, J. C., ROJAS-MOLINA, M. M., AVILA, C. M., RUBIALES, D. (2012): Induction of systemic acquired resistance against rust, ascochyta blight and broomrape in faba bean by exogenous application of salicylic acid and benzothiadiazole. *Crop Protection,* 34 65-69. p.

SPRING, O. (2001): Nonsystemic infections of sunflower with *Plasmopara halstedii* and their putative role in the distribution of the pathogen/Nichtsystemische Infektionen der Sonnenblume mit *Plasmopara halstedii* und ihre möglichen Folgen für die Ausbreitung des

2290 Pathogens. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and*
2291 *Protection*, 329-336.

2292 SPRING, O. (2009): Transition of secondary to systemic infection of sunflower with *Plasmopara*
2293 *halstedii*–An underestimated factor in the epidemiology of the pathogen. *Fungal ecology*, 2 (2)
2294 75-80. p.

2295 SPRING, O. (2019): Spreading and global pathogenic diversity of sunflower downy mildew–
2296 Review. *Plant Protection Science,* 55 (3) 149-158. p.

2297 SPRING, O., GOMEZ-ZELEDON, J., HADZIABDIC, D., TRIGIANO, R.N., THINES, M.
2298 LEBEDA, A. (2018): Biological characteristics and assessment of virulence diversity in
2299 pathosystems of economically important biotrophic oomycetes. *Critical Reviews in Plant*
2300 *Sciences,* 37 (6) 439-495. p.

2301 SPRING, O., ZIPPER, R. (2006): Evidence for asexual genetic recombination in sunflower downy
2302 mildew, *Plasmopara halstedii*. *Mycological Research,* 110 657–663. p.

2303 SPRING, O., ZIPPER, R. (2018): New highly aggressive pathotype 354 of *Plasmopara halstedii*
2304 in German sunflower fields. *Plant Protection Science,* 54 (2) 83-86. p.

2305 STEVENS, F. L. (1913): The fungi which cause plant disease (91–92). New York: Macmillan.

2306 STÖSSEL, P., LAZAROVITS, G., WARD, E. W. B. (1982): Light and electron microscopy of
2307 Phytophthora rot in soybeans treated with metalaxyl. *Phytopathology,* 72 106-111. p.

2308 TAMM, L., THÜRIG, B., FLIESSBACH, A., GOLTLIEB, A.E., KARAVANI, S., COHEN, Y.
2309 (2011): Elicitors and soil management to induce resistance against fungal plant diseases. *NJAS-*
2310 *Wageningen Journal of Life Sciences*, 58 (3-4) 131-137. p.

2311 THOMPSON, J. N., BURDON, J. J. (1992): Gene-for-Gene coevolution between plants and
2312 parasites. *Nature* 360 121–125. p.

2313 TON, J., MAUCH-MANI, B. (2004): β-amino-butyric acid-induced resistance against
2314 necrotrophic pathogens is based on ABA-dependent priming for callose. *The Plant Journal*, 38 (1)
2315 119-130. p.

2316 TOSI, L., LUIGETTI, R., ZAZZERINI, A. (1998): Induced Resistance Against *Plasmopara*
2317 *helianthi* in Sunflower Plants by DL-β-Amino-n-butyric acid. *Journal of Phytopathology*, 146 (5-
2318 6) 295-299. p.

2319 TOSI, L., ZAZZERINI, A. (2000): Interactions between *Plasmopara helianthi*, *Glomus mosseae*
2320 and two plant activators in sunflower plants. *European Journal of Plant Pathology,* 106 (8) 735-
2321 744. p.

2322 TOURVIEILLE, D. E., LABROUHE, D., BORDAT, A., TOURVIEILLE, J., MESTRIES, E.,
2323 WALSER, P., SAKR, N., DUCHER, M., DELMOTTE, F., VEAR, F. (2010): Impact of major
2324 gene resistance management for sunflower on fitness of *Plasmopara halstedii* (downy mildew)
2325 populations. *Oleagineux, Corps Gras, Lipides,* 17 (1) 56–64. p.

2326 TOURVIEILLE, D. E., LABROUHE, D., SERRE, F., ROCHE, S., WALSER, P., VEAR, F.
2327 (2008): Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus*
2328 *annuus*). *Euphytica,* 164 (2) 433–444. p.

2329 TROJANOVÁ, Z., SEDLÁŘOVÁ, M., GULYA, T. J., LEBEDA, A. (2017): Methodology of
2330 virulence screening and race characterisation of *Plasmopara halstedii*, and resistance evaluation
2331 in sunflower–a review. *Plant Pathology,* 66 (2) 171-185. p.

2332 VAJNA, L. (2002): Downy mildew epidemic on common ragweed in Hungary caused by
2333 *Plasmopara halstedii*. *Plant Pathology*, 51: 809. p.

2334 VALLAD, G. E., GOODMAN, R. M. (2004): Systemic acquired resistance and induced systemic
2335 resistance in conventional agriculture. *Crop science*, 44 (6),1920-1934. p.

2336 VAN LOON, L. C., BAKKER, P. A. H. M., PIETERSE, C. M. J. (1998): Systemic resistance
2337 induced by rhizosphere bacteria. *Annual review of phytopathology*, 36 (1) 453-483. p.

2338 VEAR, F., GENTZBITTEL, L., PHILIPPON, J., MOUZEYAR, S., MESTRIES, E., ROECKEL-
2339 DREVET, P., TOURVIEILLE DE LABROUHE, D., NICOLAS, P. (1997): The genetics of
2340 resistance to five races of downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*
2341 L.). *Theoretical and Applied Genetics*, 95 (4) 584-589. p.

2342 VEAR, F., SERIEYS, H., PETIT, A., SERRE, F., BOUDON, J. P., ROCHE, S., WALSER, P.,
2343 DE LABROUHE, D. T. (2008b, June): Origins of major genes for downy mildew resistance in
2344 sunflower. In *Proc. 17th International Sunflower Conference Cordoba, Spain, available at: www.*
2345 *isasunflower. org/publications*.

2346 VEAR, F., SERRE, F., JOUAN-DUFOURNEL, I., BERT, P.F., ROCHE, S., WALSER, P.,
2347 TOURVIEILLE DE LABROUHE, D., VINCOURT, P. (2008a): Inheritance of quantitative
2348 resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*
2349 L.). *Euphytica*, 164 (2) 561-570. p.

2350 VINCELLI, P. (2014): Some principles of fungicide resistance. *Plant Pathology Fact Sheet PPFS-*
2351 *MISC-02, University of Kentucky, College of Agriculture, Plant Pathology Extension.*

2352 VINCOURT, P., AS-SADI, F., BORDAT, A., LANGLADE, N. B., GOUZY, J., POUILLY, N.,
2353 LIPPI, Y., SERRE, F., GODIARD, L., TOURVIEILLE DE LABROUHE, D., VEAR, F. (2012):
2354 Consensus mapping of major resistance genes and independent QTL for quantitative resistance to
2355 sunflower downy mildew. *Theoretical and Applied Genetics*, 125 (5) 909-920. p.

2356 VIRÁNYI, F. (2008, June): Research progress in sunflower diseases and their management.
2357 In *Proc 17th Intl. Sunflower Conf., Cordoba, Spain* (pp. 8-12).

2358 VIRÁNYI, F., GULYA, T. J., TOURVIEILLE, D. L. (2015): Recent changes in the pathogenic
2359 variability of *Plasmopara halstedii* (sunflower downy mildew) populations from different
2360 continents. *Helia*, 38 (63) 149-162. p.

2361 VIRÁNYI, F., OROS, G. (1991): Developmental stage response to fungicides of *Plasmopara*
2362 *halstedii* (sunflower downy mildew). Mycological Research 95 (2) 199-205.

2363 VIRÁNYI, F., SPRING, O. (2011): Advances in sunflower downy mildew research. *European*
2364 *Journal of Plant Pathology,* 129 (2) 207-220. p.

2365 VIRÁNYI, F., WALCZ, I. (2000): Population studies on *Plasmopara halstedii*: host specificity
2366 and fungicide tolerance. *In Proceedings of the 15th International Sunflower Conference* 12-15.

2367 VRANCEANU, V., STOENESCU, F. (1970): Immunity to sunflower downy mildew due to a
2368 single dominant gene. *Problème Agricole,* 22 34–40. p.

2369 WALTERS, D. R., RATSEP, J., HAVIS, N. D. (2013): Controlling crop diseases using induced
2370 resistance: challenges for the future. *Journal of experimental botany,* 64 (5) 1263-1280. p.

2371 WARD, E. W. B., LAZAROVITS, G., STÖSSEL, P., BARRIE, S. D., UNWIN, C. H. (1980):
2372 Glyceollin production associated with control of Phytophthora rot of soybeans by the systemic
2373 fungicide, metalaxyl. *Phytopathology,* 70 738-740. p.

2374 WEHTJE, G., LITTLEFIELD, L. J., ZIMMER, D. E. (1979): Ultrastructure of compatible and
2375 incompatible reactions of sunflower to *Plasmopara halstedii*. *Canadian Journal of Botany*, 57 (4)
2376 315-323.

2377 YU, J. K., TANG, S., SLABAUGH, M. B., HEESACKER, A., COLE, G., HERRING, M.,
2378 SOPER, J., HAN, F., CHU, W. C., WEBB, D. M., THOMPSON, L. (2003): Towards a saturated
2379 molecular genetic linkage map for cultivated sunflower. *Crop science*, 43 (1) 367-387. p.

2380 ZHANG, Z. W., MA, G. J., ZHAO, J., MARKELL, S. G., QI, L. L. (2017): Discovery and
2381 introgression of the wild sunflower-derived novel downy mildew resistance gene *Pl 19* in
2382 confection sunflower (*Helianthus annuus* L.). *Theoretical and applied genetics*, 130 (1) 29-39. p.

2383

2384

2385

2386

2387

2388

2389

2390

2391

2392

2393

2394

2395

2396

2397

2398

2399

2400

2401

2402

2403

2404

2405

2406

2407

2408

2409

2410

2411 **Appendix 1.** Data of *Plasmopara halstedii* isolates used in the experiments of the thesis

Isolate ID in MATE collection (year, month, day, number, country)	**Locality**	**Code of isolate for pathotype identification**	**Code of isolate for 10-isolate experiment**	**Code of isolate for 8-isolate experiment**	**Code of isolate for 5-isolate experiment**	**Pathotype (CVF*) of isolate, new result in dissertation (CVF published before, not new in thesis)**
Ph-20170613-23/1-Hu	**Karácsond**	1	I4	-	-	704
Ph-20170523-2/1-Hu	Martfű	2	-	-	-	704
Ph-20170609-18/1-Hu	Galgahévíz	3	-	-	-	704
Ph-20170621-28/1-Hu	Csongrád	4	-	-	-	704
Ph-20160621-5/1B-Hu	Csongrád	-	I7	-	-	(704)
Ph-20170529-4/1-Hu	Hatvan	5	-	-	-	700
Ph-20170529-4/2-Hu	Hatvan	6	-	-	-	704
Ph-2017070	Pély	7	I6	8	-	704

3-40/1-Hu						
Ph-20170613-22/1-Hu	Túrkeve	8	I3	-	-	700
Ph-20170622-29/C1-Hu	Bonyhád	9	I5	9	-	724
Ph-20170622-29/B-Hu	Bonyhád	10	-	-	-	704
Ph-20170606-15/B-Hu	Vésztő	11	-	-	-	724
Ph-20170628-31/1-Hu	**Szeged**	12	-	-	-	714
Ph-20170601-12/1-Hu	**Abony**	13	-	-	-	704
Ph-20170530-7/1-Hu	Tápé	14	-	-	-	704
Ph-20170630-34/A-Hu	Szamoskér	15	-	-	-	700
Ph-20180601-4/1-Hu	unknown (Hungary)	16	-	-	-	700
Ph-20190522-7/3-Hu	**Békés-szentandrás**	17	-	-	-	724

Ph-20190627-21/1-Hu	Léh	18	-	-	-	734
Ph-20190606-14/1-Hu	**Bucsa**	19	-	-	-	734
Ph-20190606-14/3-Hu	Kertészsziget	20	-	-	-	734
Ph-20190606-14/4-Hu	Kötegyán	21	-	-	-	730
Ph-20190618-18/2-Hu	**Vanyarc**	22	-	-	-	734
Ph-20170608-16/1B-Hu	Mezőkovácsháza	-	I2	1	-	(724)
Ph-20140626-23/1-Hu	Kömlő	-	-	4	Kömlő	(704)
Ph-20140527-9/1-Hu	Doboz	-	-	5	-	(704)
Ph-20140527-7/2-Hu	Köröstladány	-	-	6	-	(714)
Ph-20140527-7/1-Hu	Köröstladány	-	I9	-	-	(704)
Ph-2017060	Szeghalom	-	-	7	-	(724)

8-17/1C-Hu						
Ph-20120626-7/1-Hu	Rákóczifalva	-	-	11	Rákóczi-falva	(704)
Ph-20170523-1/1	Tiszaföldvár	-	I1	-	-	(704)
Ph-20140521-6/1-Hu	Tiszafüred	-	I8	-	-	(730)
Ph-20140611-11/1-Hu	Csanytelek	-	I10	-	Csanytelek	(730)
Ph-20140521-5/1-Hu	Mád (1,2)	-	-	-	Mád1, Mád2	(700)