Research

The Diversification of Downy Mildew Species Was Not Driven by the Loss of Mycorrhizal Associations or the Evolution of C₄ Photosynthesis

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Abstract

There are approximately 700 obligate biotrophic species grouped into 20 genera (Oomycota, Peronosporaceae) that cause downy mildew diseases. In 2001, Dick hypothesized that diversification of downy mildew species was driven in part by host plant secondary metabolites. Dick further speculated that this was driven by the transition of host plants away from mycorrhizal associations or the evolution of C₄ photosynthesis. Specifically, loss of mycorrhizal associations or the use of C₄ photosynthesis would result in more free carbon that the plants could then use to produce more secondary metabolites. If true, then there should be more downy mildew species that infect hosts from plant lineages that lack mycorrhizal associations or use C₄ photosynthesis. However, analysis of 677 downy mildew species for host plant mycorrhizal associations and host plant photosynthetic pathway type shows that this is not what occurred. Seventy percent of downy mildew species parasitize hosts that form mycorrhizal associations, and 94% of downy mildew species parasitize hosts that use C₃ photosynthesis. From this, it is concluded that the diversification of downy mildew species was not driven by the loss of mycorrhizal associations or the evolution of C₄ photosynthesis. However, 85% of downy mildew species that parasitize Poaceae (grasses) parasitize C₄ hosts. Thus, it is possible that C₄ photosynthesis plays a role in the diversification of these genera.

Keywords: biodiversity, graminicolous downy mildews, *Hyaloperonospora*, *Peronospora*, *Peronosclerospora*, *Plasmopara*, *Sclerophthora*, *Sclerospora*

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Downy mildew diseases are caused by ~700 obligate biotrophic species grouped into 20 oomycete genera within the Peronosporaceae, often referred to collectively as downy mildews (Thines and Choi 2016). Downy mildew genera are nested within the genus *Phytophthora* and represent at least two evolutionary transitions from hemitrophy to obligate biotrophy (Bourret et al. 2018; Fletcher et al. 2019; McCarthy and Fitzpatrick 2017; Thines and Choi 2016). Thus, *Phytophthora* and the downy mildews represent a unique opportunity to study the molecular underpinnings of trophic shifts and the convergence of traits. Furthermore, the diversity within the downy mildew genera seems

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to be driven by host jumps and radiation rather than strict co-phylogenetic evolution (Dick 2001; Thines 2014; Thines and Choi 2016). Therefore, the diversity within downy mildew genera also offers a unique opportunity to study the molecular underpinnings of host jumps and the selective forces that lead to host specificity and speciation. This is particularly true for species of *Peronosclerospora* that parasitize maize because maize is not predicted to be the natural host of these species (Shaw 1975; Thines 2014). However, the relationships among and within the genera remain unresolved owing to low taxon sampling and low sampling of loci (Thines and Choi 2016), which hampers investigations into the evolution of traits and lifestyles.

Even in the absence of resolved relationships, there are hypotheses about the evolution of downy mildew species that can be evaluated. For example, Dick (2001) hypothesized that the radiation of downy mildew species would be facilitated/driven by the secondary metabolism of their host plants and listed a variety of correlations as evidence in support of this hypothesis. As part of his hypothesis, Dick (2001) speculated that host plant species lacking mycorrhizal associations have more carbon to produce secondary metabolites, which in turn makes such host plant species attractive from the perspective of a downy mildew species. If true, then one would expect there to be more downy mildew species on host plants that lack mycorrhizal associations than on host plants that form mycorrhizal associations. Dick (2001) expected this to be particularly true for species of *Peronospora* and *Hyaloperonospora* due to their diversity on plant species in Caryophyllaceae and Brassicales, respectively. However, Dick (2001) did not formally test these hypotheses, nor have later authors.

Approximately 90% of plant families form mycorrhizal associations, which aid the host in water and nutrient acquisition. There are four main types of mycorrhizas: arbuscular mycorrhizas, ectomycorrhizas, ericoid mycorrhizas, and orchid mycorrhizas (Brundrett and Tedersoo 2018). Approximately 72% of flowering plant species form associations with arbuscular mycorrhizas, and it is believed that these associations evolved early in the history of land plants (Brundrett and Tedersoo 2018). In fact, it is generally thought that nonmycorrhizal plant lineages are an evolutionary loss of the arbuscular mycorrhizal association (Brundrett and Tedersoo 2018). This hypothesis is supported by the presence of genes necessary for the formation of arbuscular mycorrhizal associations in some nonmycorrhizal plants (Ried et al. 2019).

Dick (2001) also speculated that host plants that use C₄ photosynthesis would be favored by downy mildew species for the same reason that downy mildew species might favor nonmycorrhizal hosts (i.e., increased carbon for secondary metabolite production). Spencer and Dick (2002) further hypothesized that this relationship would be most prominent with species that cause downy mildews on plant hosts in Poaceae (i.e., grasses). There have been at least 61 independent evolutions of C₄ photosynthesis (Sage 2016). The largest number of C₄ species (5,044) is within the Poaceae (Sage 2016). In comparison, there are 1,777 eudicot species that use the C₄ photosynthetic pathway, with the greatest numbers in Amaranthaceae s.l. (815 species) and Euphorbiaceae (350 species; Sage 2016). In general, plant species that use C₄ photosynthesis are better adapted to warm climates (Sage 2016). As with the hypothesized relationship between mycorrhizal status, secondary metabolism, and diversification of downy mildew species, the hypothesized relationship between the evolution of C₄ photosynthesis and the diversification of downy mildew species has not been tested.

Herein, the hypotheses that (i) the lack of mycorrhizal associations would facilitate the diversification of downy mildew species

and (ii) the C₄ photosynthesis pathway would facilitate diversification of downy mildew species were evaluated. It was predicted that if these hypotheses were true, then (i) there would be a greater number of downy mildew species on nonmycorrhizal host plant species than on mycorrhizal host plant species and that (ii) there would be a greater number of downy mildew species on C₄ host plant species than on C₃ host plant species.

METHODS

A list of downy mildew species and their type host plant species was generated through an intensive literature search. Species names of both the pathogen and the host were hand-coded into an Excel spreadsheet using monographic works (Barretto and Dick 1991; Constantinescu 1989, 1991, 1992, 1998, 2004; Constantinescu and Fatehi 2002; Constantinescu et al. 2005; Voglmayr et al. 2014a; Waterhouse 1964; Waterhouse and Brothers 1981), Index Fungorum (<http://www.indexfungorum.org/>), the original descriptions, and later descriptions (see Supplementary References). For species described prior to 1958 that had multiple hosts listed in the description and have not since been lectotypified (e.g., *Peronospora aestivalis*; Constantinescu 1991), the first host species listed (e.g., *Medicago sativa*; Constantinescu 1991) was used as the type host species. Analyses were restricted to the type host/a single host to avoid perpetuating potential errors in identification (e.g., the reports of *Sclerophthora cryophila* on *Heteropogon contortus* and *Dichanthium annulatum* [Farr and Rossman 2021] that are likely *Peronosclerospora heteropogonis* and *Peronosclerospora dichanthiicola*, respectively, which are species described after the initial reports were made) and potential pseudoreplication. Such an approach is justified because most phylogenetically defined downy mildew species have a narrow plant host range with either one host or a few closely related hosts (e.g., Choi et al. 2008, 2009a, 2009b, 2010, 2015, 2020; Cunningham 2006; García-Blázquez et al. 2008; Görg et al. 2017; Petrželová et al. 2017; Rouxel et al. 2014; Runge and Thines 2012; Runge et al. 2011; Thines 2011; Thines and Choi 2016; Voglmayr et al. 2014b). *Peronospora* species infecting hosts in the Brassicales were considered to be members of *Hyaloperonospora*, even in the absence of formal transfers, based on the trends observed by Constantinescu and Fahtei (2002), Göker et al. (2003, 2004, 2009a, 2009b), Voglmayr et al. (2014a), and Salgado-Salazar et al. (2020). Yang and Berry (2011), Kadereit et al. (2003, 2010), Riina et al. (2013), and Sage (2016) were consulted to determine what photosynthetic pathway each of the plant hosts used. This information was then manually added to the Excel spreadsheet. The spreadsheet was then imported into R 4.0.3 (R Core Team 2020). Using the packages found in the tidyverse (Wickham et al. 2019), column names were converted to snakecase, extra columns were removed, the columns were reordered, and the mycorrhizal status of the host plant species was coded based on the mycorrhizal status of the host plant family according to Brundrett (2008, 2009). For the purposes of this study, no distinction was made between the different kinds of mycorrhizal associations or different forms of C₄ photosynthesis. The number of species in each downy mildew genus that infected a plant host with/without mycorrhiza was tabulated. The number of species in each downy mildew genus that infected plant hosts using C₃, Crassulacean acid metabolism, or C₄ photosynthetic pathways was tabulated. Graphs were generated using ggplot2 (Wickham 2016), RColorBrewer (Neuwirth 2014), and patchwork (Pedersen 2021). Because the data set (i.e., the list of species, typical host plant, photosynthetic pathway, and status of mycorrhizal association) comprises the entire population (i.e., is not a sample), the use of sample-based statistical tests (e.g., chi-

squared test) is not appropriate because the exact values (rather than estimates) are known, and nothing needs to be inferred (Quinn and Keough 2002). Data, scripts, and output are available on GitHub (https://github.com/wjdavis90/Downy_mildews/tree/main/DM_evolution).

RESULTS

The majority of plant hosts infected by downy mildews form mycorrhizal associations, with 70% of the 677 downy mildew species described from mycorrhizal hosts (Fig. 1). Two genera—*Hyaloperonospora* (95 species) and *Perofascia* (two species)—were associated exclusively with nonmycorrhizal hosts. Only two genera had species with mycorrhizal and nonmycorrhizal plant hosts: *Peronospora* had 277 species on mycorrhizal plant hosts and 104 species on nonmycorrhizal plant hosts. *Plasmopara* had 118 species on mycorrhizal plant hosts and four species on nonmycorrhizal plant hosts.

The majority of downy mildew species (94%) infected plant hosts that utilize the C_3 photosynthesis pathway (Fig. 2). *Peronospora* had 367 species on plant hosts that use C_3 photosynthesis, one species on a plant host that can switch between C_3 and Crassulacean acid metabolism pathways, and 13 species on plant hosts that use C_4 photosynthesis. *Plasmopara* and *Hyaloperonospora* species were only found on plants that use C_3 photosynthesis. For downy mildew parasites of Poaceae, 24 of the 28 species were parasites of grasses that

use C_4 photosynthesis (Fig. 2B). All the species in *Baobabopsis*, *Erapthora*, *Peronosclerospora*, *Poakateshia*, and *Viennotia* were on C_4 grasses. *Sclerophthora* had four species on C_3 grasses and two species on C_4 grasses. *Sclerospora* had three species on C_4 grasses and one species on a C_3 host plant.

DISCUSSION

Dick (2001) hypothesized that the production of secondary metabolites by host plants facilitated the diversification of downy mildew species. As part of this hypothesis, he speculated that lack of mycorrhizal associations would lead to more available carbon and therefore more secondary metabolites. This in turn would make such plants more favorable as hosts for downy mildew species. If true, this should result in a greater diversification of downy mildew species on plant hosts that lack mycorrhizal associations. However, the observed patterns of species diversity do not support this. More downy mildews were found on host plants that form mycorrhizal associations than on host plants that do not. The two exceptions were the genera *Hyaloperonospora* and *Perofascia*, in which all species parasitized host plants in Brassicales, which is a nonmycorrhizal order of plants (Brundrett 2008, 2009; Delaux et al. 2014). Because there is less species diversity in *Hyaloperonospora* than in *Peronospora* and *Plasmopara*, it seems unlikely that the diversification was due to the lack of mycorrhizal associations. Thus, it is concluded that downy mildew

FIGURE 1 Distribution of downy mildew species among mycorrhizal and nonmycorrhizal hosts in the three largest downy mildew genera (A) and in the other 17 genera (B).

FIGURE 2 Distribution of downy mildew species among C_3 and C_4 photosynthetic hosts in the three largest downy mildew genera (A) and in the other 17 genera (B). Downy mildew genera that parasitize species of Poaceae (grasses) are in bold.

species did not evolve to favor host plant species that lack mycorrhizal associations.

The description of additional downy mildew species due to the resolution of known species complexes (e.g., *Plasmopara halstedii* and *Pl. viticola*) will not change the observed patterns of diversity or the above conclusion. The cryptic lineages within these species complexes are associated with closely related plant species, often in the same genus (e.g., *Vitis* spp.; Rouxel et al. 2013, 2014) or family (e.g., Asteraceae; Rivera et al. 2016). Thus, the plant hosts share the same mycorrhizal or nonmycorrhizal status. The underlying numbers may change, but the observed pattern of a greater number of downy mildew species on mycorrhizal plant hosts will not.

A hypothesis that Dick (2001) did not consider is that the loss of mycorrhizal associations might make it easier for a downy mildew species to jump between distantly related host lineages because of overall lower plant immunity and/or secondary metabolites. The presence of a mycorrhizal association has been found to enhance the host plant's immune system and the production of secondary metabolites (e.g., Avio et al. 2017; Bruisson et al. 2016; Cruz-Silva et al. 2021; Pistelli et al. 2017; Wang et al. 2017). In other words, loss of mycorrhizal associations in some plant lineages might have facilitated host jumps (but not necessarily diversification) because nonmycorrhizal plant species potentially offer a lower barrier to survival if the "wrong" host is encountered. However, this is untestable at present because the direction of host jumps in relation to mycorrhizal status cannot be determined without a sufficiently resolved downy mildew species phylogeny.

Dick (2001) also speculated that the use of C₄ photosynthesis would lead to more available carbon and more secondary metabolites, which would make such plants more favorable hosts for downy mildew species. If true, this should result in a greater diversification of downy mildew species on plant hosts that use C₄ photosynthesis. However, with 94% of all downy mildew species associated with plants that use C₃ photosynthesis, the observed patterns of species diversity do not support this hypothesis. Thus, it is concluded that downy mildew species did not evolve to favor plant host species that use C₄ photosynthesis.

In line with Spencer and Dick's (2002) hypothesis, in downy mildew genera that parasitize Poaceae—*Baobabopsis*, *Erapthora*, *Peronosclerospora*, *Poakatesthia*, *Sclerophthora*, *Sclerospora*, and *Viennotia*—there were more species that infect grasses that use the C₄ pathway (24 species) than the C₃ pathway (four species). All the species in *Baobabopsis*, *Erapthora*, *Peronosclerospora*, *Poakatesthia*, and *Viennotia* infect C₄ grasses. All species of *Sclerospora* that infect grasses are on C₄ hosts. *Sclerospora magnusiana* parasitizes the C₃ host *Equisetum arvense*, but it is a dubious member of the genus (Waterhouse 1964). One notable deviation is the genus *Sclerophthora*, which contains four species on C₃ grasses and two species on C₄ grasses.

However, the higher diversity of downy mildew species on C₄ grasses than on C₃ grasses can be explained by means other than secondary metabolites. One alternative explanation for the observed pattern is sampling bias; specifically, most of the known downy mildew species infect economically important grasses, which are disproportionately C₄ species (Sage 2016). Another hypothesis is that C₄ grasses are adapted to the same environmental conditions that favor growth of downy mildew species (i.e., warm, moist climates). The data at hand cannot distinguish between Spencer and Dick's (2002) hypothesis and these alternative hypotheses of sampling bias or correlation of favorable environmental conditions. Thus, Spencer and Dick's (2002) hypothesis cannot be accepted or rejected at this time.

The description of additional downy mildew species due to the resolution of known species complexes (e.g., *Sclerospora*

graminicola) will not change the observed patterns of diversity or the above conclusions. The cryptic lineages within these species complexes tend to be on hosts in the same subfamily. Thus, the plant hosts share the same type of photosynthesis pathway. The underlying numbers may change, but the pattern of a greater number of downy mildew species on C₄ grasses will not.

The results presented herein do not mean that Dick's (2001) higher hypothesis that secondary metabolites play an important role in the evolution and diversification of downy mildew species is rejected. Rather, what is rejected is the proposed mechanism that lack of mycorrhizal association or use of the C₄ photosynthetic pathways increases the production of secondary metabolites and therefore makes certain plant lineages better hosts for downy mildew species. However, it is still possible that the amounts and types of secondary metabolites in a host plant lineage drive evolution and diversification, especially in cases of host jumps (Dick 2001; Thines 2014; Thines and Choi 2016).

In summary, there are more downy mildew species associated with plant hosts that form mycorrhizal associations or C₃ plant species than plant hosts that do not form mycorrhizal associations or C₄ plant species. Thus, it is concluded that, in general, downy mildew species did not evolve to favor plant host species that lack mycorrhizal associations or that use C₄ photosynthesis.

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LITERATURE CITED

- Avio, L., Sbrana, C., Giovannetti, M., and Frassinetti, S. 2017. Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. *Sci. Hortic.* 224:265-271.
- Barreto, R. W., and Dick, M. W. 1991. Monograph of Basidiophora (Oomycetes) with the description of a new species. *Bot. J. Linn. Soc.* 107:313-332.
- Bourret, T. B., Choudhury, R. A., Mehl, H. K., Blomquist, C. L., McRoberts, N., and Rizzo, D. M. 2018. Multiple origins of downy mildews and mitochondrial discordance within the paraphyletic genus *Phytophthora*. *PLoS ONE* 13:e0192502.
- Bruisson, S., Maillot, P., Schellenbaum, P., Walter, B., Gindro, K., and Deglène-Benbrahim, L. 2016. Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection. *Phytochemistry* 131:92-99.
- Brundrett, M. C. 2008. Mycorrhizal Associations: The Web Resource. <https://mycorrhizas.info>.
- Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320:37-77.
- Brundrett, M. C., and Tedersoo, L. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* 220:1108-1115.
- Choi, Y.-J., Denchy, C. M., and Shin, H.-D. 2008. Morphological and molecular analyses support the existence of host-specific *Peronospora* species infecting *Chenopodium*. *Mycopathologia* 165:155.
- Choi, Y.-J., Kiss, L., Vajna, L., and Shin, H.-D. 2009a. Characterization of a *Plasmopara* species on *Ambrosia artemisiifolia*, and notes on *P. halstedii*, based on morphology and multiple gene phylogenies. *Mycol. Res.* 113:1127-1136.
- Choi, Y.-J., Shin, H.-D., and Thines, M. 2009b. Two novel *Peronospora* species are associated with recent reports of downy mildew on sages. *Mycol. Res.* 113:1340-1350.

- Choi, Y.-J., Danielsen, S., Lübeck, M., Hong, S.-B., Delhey, R., and Shin, H.-D. 2010. Morphological and molecular characterization of the causal agent of downy mildew on quinoa (*Chenopodium quinoa*). *Mycopathologia* 169:403-412.
- Choi, Y.-J., Klosterman, S. J., Kummer, V., Voglmayr, H., Shin, H.-D., and Thines, M. 2015. Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (Straminipila, Oomycota), including pathogens of beet and spinach. *Mol. Phylogenet. Evol.* 86:24-34.
- Choi, Y.-J., Görg, M., Shin, H.-D., and Thines, M. 2020. *Plasmopara elegantissima* sp. nov. (Oomycota, Peronosporales), a downy mildew species specialized to *Impatiens textori* (Balsaminaceae). *Mycobiology* 48: 304-312.
- Constantinescu, O. 1989. *Peronospora* complex on Compositae. *Sydowia* 41:79-107.
- Constantinescu, O. 1991. An annotated list of *Peronospora* names. *Thunbergia* 15:1-110.
- Constantinescu, O. 1991. *Bremiella sphaerosperma* sp. nov. and *Plasmopara borrieriae* comb. nov. *Mycologia* 83:473-479.
- Constantinescu, O. 1992. The nomenclature of *Plasmopara* parasitic on Umbelliferae. *Mycotaxon* 43:471-477.
- Constantinescu, O. 1998. A revision of *Basidiophora* (Chromista, Peronosporales). *Nova Hedwigia* 66:251-265.
- Constantinescu, O. 2004. The nomenclature of *Plasmopara* (Chromista, Peronosporales) parasitic on Geraniaceae. *Taxon* 53:523-525.
- Constantinescu, O., and Fatehi, J. 2002. *Peronospora*-like fungi (Chromista, Peronosporales) parasitic on Brassicaceae and related hosts. *Nova Hedwigia* 74:291-338.
- Constantinescu, O., Voglmayr, H., Fatehi, J., and Thines, M. 2005. *Plasmoverna* gen. nov., and the taxonomy and nomenclature of *Plasmopara* (Chromista, Peronosporales). *Taxon* 54:813-821.
- Cruz-Silva, A., Figueiredo, A., and Sebastiana, M. 2021. First insights into the effects of mycorrhizae on the expression of pathogen effectors during the infection of grapevine with *Plasmopara viticola*. *Sustainability* 13: 1226.
- Cunnington, J. H. 2006. DNA sequence variation supports multiple host-specialised taxa in the *Peronospora viciae* complex (Chromista: Peronosporales). *Nova Hedwigia* 82:23-29.
- Delaux, P.-M., Varala, K., Edger, P. P., Coruzzi, G. M., Pires, J. C., and Ané, J.-M. 2014. Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. *PLoS Genet.* 10:e1004487.
- Dick, M. W. 2001. Straminipilous Fungi: Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protists, the Plasmodiophorids and Similar Organisms, 1st Ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Farr, D. F., and Rossman, A. Y. 2021. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. <https://nt.ars-grin.gov/fungal-databases/>.
- Fletcher, K., Gil, J., Bertier, L. D., Kenefick, A., Wood, K. J., Zhang, L., Reyes-Chin-Wo, S., Cavanaugh, K., Tsuchida, C., Wong, J., and Michelmore, R. 2019. Genomic signatures of heterokaryosis in the oomycete pathogen *Bremia lactucae*. *Nature* 10:2645.
- García-Blázquez, G., Göker, M., Voglmayr, H., Martín, M. P., Tellería, M., and Oberwinkler, F. 2008. Phylogeny of *Peronospora*, parasitic on Fabaceae, based on ITS sequences. *Mycol. Res.* 112:502-512.
- Göker, M., García-Blázquez, G., Voglmayr, H., Tellería, M. T., and Martín, M. P. 2009b. Molecular taxonomy of phytopathogenic fungi: A case study in *Peronospora*. *PLoS ONE* 4:e6319.
- Göker, M., Riethmüller, A., Voglmayr, H., Weiß, M., and Oberwinkler, F. 2004. Phylogeny of *Hyaloperonospora* based on nuclear ribosomal internal transcribed spacer sequences. *Mycol. Prog.* 3:83-94.
- Göker, M., Voglmayr, H., García-Blázquez, G., and Oberwinkler, F. 2009a. Species delimitation in downy mildews: The case of *Hyaloperonospora* in light of nuclear ribosomal ITS and LSU sequences. *Mycol. Res.* 113: 308-325.
- Göker, M., Voglmayr, H., Riethmüller, A., Weiß, M., and Oberwinkler, F. 2003. Taxonomic aspects of *Peronosporaceae* inferred from Bayesian molecular phylogenetics. *Can. J. Bot.* 81:672-683.
- Görg, M., Ploch, S., Kruse, J., Kummer, V., Runge, F., Choi, Y.-J., and Thines, M. 2017. Revision of *Plasmopara* (Oomycota, Peronosporales) parasitic to *Impatiens*. *Mycol. Prog.* 16:791-799.
- Kadereit, G., Borsch, T., Weising, K., and Freitag, H. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C₄ photosynthesis. *Int. J. Plant Sci.* 164:959-986.
- Kadereit, G., Mavrodiev, E. V., Zacharias, E. H., and Sukhorukov, A. P. 2010. Molecular phylogeny of Atripliceae (Chenopodiaceae, Chenopodiaceae): Implications for systematics, biogeography, flower, and fruit evolution, and the origin of C₄ photosynthesis. *Am. J. Bot.* 97:1664-1687.
- McCarthy, C. G. P., and Fitzpatrick, D. A. 2017. Phylogenomic reconstruction of the oomycete phylogeny derived from 37 genomes. *mSphere* 2: e00095-17.
- Neuwirth, E. 2014. RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. <https://CRAN.R-project.org/package=RColorBrewer>.
- Pedersen, T. L. 2021. Patchwork: The composer of plots. <https://patchwork.data-imaginist.com>, <https://github.com/thomasp85/patchwork>.
- Petrželová, I., Choi, Y.-J., Jemelková, M., Doležalová, I., Kruse, J., Thines, M., and Kitner, M. 2017. Confirmation of *Peronospora agrimoniae* as a distinct species. *Eur. J. Plant Pathol.* 147:887-896.
- Pistelli, L., Uliviera, V., Giovannelli, S., Avio, L., Giovannetti M., Pistelli, L. 2017. Arbuscular mycorrhizal fungi alter the content and composition of secondary metabolites in *Bituminaria bituminosa* L. *Plant Biol.* 19: 926-933.
- Quinn, G. P., and Keough, M. J. 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, U.K.
- R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Ried, M. K., Banhara, A., Hwu, F.-Y., Binder, A., Gust, A. A., Höfle, C., Hückelhoven, R., Nürnberger, T., and Parniske, M. 2019. A set of *Arabidopsis* genes involved in the accommodation of the downy mildew pathogen *Hyaloperonospora arabidopsidis*. *PLoS Pathog.* 15:e1007747.
- Riina, R., Peirson, J. A., Geltman, D. V., Molero, J., Frajman, B., Pahlevani, A., Barres, L., Morawetz, J. J., Salmaki, Y., Zarre, S., Kryukov, A., Bruyns, P. V., and Berry, P. E. 2013. A worldwide molecular phylogeny and classification of the leafy spurges, *Euphorbia* subgenus *Esula* (Euphorbiaceae). *Taxon* 62:316-342.
- Rivera, Y., Salgado-Salazar, C., Gulya, T. J., and Crouch, J. A. 2016. Newly emerged populations of *Plasmopara halstedii* infecting *Rubeckia* exhibit unique genotypic profiles and are distinct from sunflower-infecting strains. *Phytopathology* 106:752-761.
- Rouxel, M., Mestre, P., Baudoin, A., Carisse, O., Delière, L., Ellis, M. A., Gadoury, D., Lu, J., Nita, M., Richard-Cervera, S., Schilder, A., Wise, A., and Delmotte, F. 2014. Geographic distribution of cryptic species of *Plasmopara viticola* causing downy mildew on wild and cultivated grape in Eastern North America. *Phytopathology* 104:692-701.
- Rouxel, M., Mestre, P., Comont, G., Lehman, B. L., Schilder, A., and Delmotte, F. 2013. Phylogenetic and experimental evidence for host-specialized cryptic species in a biotrophic oomycete. *New Phytol.* 197: 251-263.
- Runge, F., Choi, Y.-J., and Thines, M. 2011. Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. *Eur. J. Plant Pathol.* 129:135-146.
- Runge, F., and Thines, M. 2012. Reevaluation of host specificity of the closely related species *Pseudoperonospora humuli* and *P. cubensis*. *Plant Dis.* 96:55-61.
- Sage, R. F. 2016. A portrait of the C₄ photosynthetic family on the 50th anniversary of its discovery: Species number, evolutionary lineages, and hall of fame. *J. Exp. Bot.* 67:4039-4056.
- Salgado-Salazar, C., LeBlanc, N., Wallace, E. C., Daughtrey, M. L., and Crouch, J. A. 2020. *Peronospora monardae*, *Hyaloperonospora daughtreyae*, and *H. iberidis*: New species associated with downy mildew diseases affecting ornamental plants in the United States. *Eur. J. Plant Pathol.* 157:311-326.
- Shaw, C. G. 1975. The taxonomy of graminicolous downy mildews, with emphasis on those attacking maize. *Trop. Res. Ser.* 8:47-55.
- Spencer, M. A., and Dick, M. W. 2002. Aspects of graminicolous downy mildew biology: Perspectives for tropical plant pathology and *Peronosporomycetes* phylogeny. Pages 63-81 in: *Tropical Mycology*, Vol. 2. R. Watling, ed. CABI Publishing, Wallingford, U.K.
- Thines, M. 2011. Recent outbreaks of downy mildew on grape ivy (*Parthenocissus tricuspidata*, Vitaceae) in Germany are caused by a new species of *Plasmopara*. *Mycol. Prog.* 10:415-422.
- Thines, M. 2014. Phylogeny and evolution of plant pathogenic oomycetes—A global overview. *Eur. J. Plant Pathol.* 138:431-447.
- Thines, M., and Choi, Y.-J. 2016. Evolution, diversity, and taxonomy of the *Peronosporaceae*, with focus on the genus *Peronospora*. *Phytopathology* 106:6-18.
- Voglmayr, H., Choi, Y.-J., and Shin, H.-D. 2014a. Multigene phylogeny, taxonomy, and reclassification of *Hyaloperonospora* on *Cardamine*. *Mycol. Prog.* 13:131-144.
- Voglmayr, H., Montes-Borrego, M., and Landa, B. B. 2014b. Disentangling *Peronospora* on *Papaver*: Phylogenetics, taxonomy nomenclature and host range of downy mildew of opium poppy (*Papaver somniferum*) and related species. *PLoS ONE* 9:e96838.
- Wang, Y.-Y., Yin, Q.-S., Qu, Y., Li, G.-Z., and Hao L. 2017. Arbuscular mycorrhiza-mediated resistance in tomato against *Cladosporium fulvum*-induced mould disease. *J. Phytopathol.* 166:67-74.
- Waterhouse, G. M. 1964. The genus *Sclerospora*: Diagnoses (or descriptions) from the original papers and a key. *Comm. Mycol. Inst. Misc. Pub.* 17: 1-30.

- Waterhouse, G. M., and Brothers, M. P. 1981. The taxonomy of *Pseudoperonospora*. Mycol. Papers 148:1-28.
- Wickham, H. 2016. Ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York, NY.
- Wickham, H., Averick, M., Ryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., Takahashi, K., Vaughn, D., Wilke, C., Woo, K., and Tutani, H. 2019. Welcome to the tidyverse. J. Open Source Software 4:1686.
- Yang, Y., and Berry, P. E. 2011. Phylogenetics of the Chamaesyce clade (*Euphorbia*, Euphorbiaceae): Reticulate evolution and long-distance dispersal in a prominent C₄ lineage. Am. J. Bot. 98:1486-1503.