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► To cite this version:

Benjamin Petre, Sébastien Duplessis. A decade after the first Pucciniales genomesa snapshot of (post) genomics studies in three model rust fungi. 2022. hal-03719656

HAL Id: hal-03719656

<https://hal.science/hal-03719656>

Preprint submitted on 11 Jul 2022

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A decade after the first Pucciniales genomes – a snapshot of (post) genomics studies in three model rust fungi

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6

7 Abstract

8 Pucciniales (Rust fungi) are one of the largest fungal order of plant pathogens. They collectively infect
9 key crops such as wheat and soybean, and threaten global food security. In the early 2010s, the genome
10 sequences of three rust fungi were released: *Melampsora larici-populina* (MLP; the poplar leaf rust
11 fungus), *Puccinia graminis* f. sp. *tritici* (PGT; the wheat stem rust fungus), and *Puccinia striiformis* f.
12 sp. *tritici* (PST; the wheat stripe rust or wheat yellow rust fungus). The availability of those genomes
13 has forwarded rust biology into the post-genomic era, sparking series of genomics, transcriptomics, *in*
14 *silico*, and functional studies. Here, we snapshot the last ten years of post-genomics studies addressing
15 MLP, PGT, and/or PST. The mini-review notably reveals the model species-centered structuration of
16 the research community, and highlights the drastic increase of the number of functional studies focused
17 on effectors since 2014, which notably revealed chloroplasts as a central host compartment targeted by
18 rust fungi. The mini-review also discusses genomics-facilitated studies in other rust species, and
19 emerging post-genomic research trends related to fully-phased rust genomes.

20 Keywords

21 Fungal parasite, plant disease, transcriptome, basidiomycete, microbe, bibliometric analysis,
22 virulence protein, plant pathology

23

24 Main Text

25 Introduction

26 Rust fungal pathogens infect key crops and threaten global food security. Despite their importance, the
 27 research community understudies them, mostly due to technical limitations associated with the lack of
 28 reverse genetics tools. Rust fungi belong to the order Pucciniales; one of the largest fungal pathogen
 29 order (Aime and McTaggart, 2021). Pucciniales can infect a wide variety of host plants, and attract a
 30 particular attention in the phytopathology community because they impact major crops worldwide
 31 (Moscou and van Esse, 2017). Rust fungi are obligate biotrophs, have a complex lifecycle, and do not
 32 infect some model plants; so far, those features have precluded the establishment of routine approaches
 33 for reverse genetics and have pushed the research community to use heterologous plant systems
 34 (Lorrain et al., 2018; Duplessis et al., 2021). In the late 2000s, the rust biology research community
 35 acknowledged the necessity to quickly obtain the first rust genome sequences, and jointed efforts on
 36 key model rust species (Duplessis et al., 2014; Aime et al., 2017).

37 In the early 2010s, three publications reported the first genome sequences of three rust fungi:
 38 *Melampsora larici-populina* (MLP; the poplar leaf rust fungus), *Puccinia graminis* f. sp. *tritici* (PGT;
 39 the wheat stem rust fungus), and *Puccinia striiformis* f. sp. *tritici* (PST; the wheat stripe rust or wheat
 40 yellow rust fungus) (Cantu et al., 2011; Duplessis et al., 2011; Zheng et al., 2013). MLP and PGT
 41 genomes were obtained through first generation sequencing technology (*i.e.*, Sanger sequencing),
 42 whereas PST genome sequences were obtained through either first or second generation sequencing
 43 technologies (*i.e.*, Sanger and/or Illumina) (Duplessis et al., 2014). Although these pioneer attempts
 44 led to various levels of quality of genome assembly and annotation, they revealed unique features
 45 among fungal biotrophs, which has been proven true since then: rust fungi possess large genomes, with
 46 a substantial content in repeat elements (*e.g.*, transposable elements) and with expanded gene families
 47 and total number of predicted genes (Duplessis et al., 2013; Aime et al., 2017). The availability of those
 48 genome sequences has forwarded rust biology into the post-genomic era, sparking series of studies
 49 that addressed fundamental questions related to the evolution, biology, and virulence mechanisms of
 50 rust fungi (Figueroa et al., 2016; Lorrain et al., 2019).

51 This minireview provides readers with an overview of (post) genomic researches that addressed MLP,
 52 PGT, or PST, by identifying and analyzing 82 Rust (Post) Genomics publications (hereafter ‘RPGs’)
 53 published between 2010 and 2021. We first snapshot the collection of RPGs and associated publication
 54 metrics. Then, we show how the first genomics studies facilitated both a wave of functional analyses
 55 of candidate effectors proteins (hereafter ‘effectors’) as well as studies in other rust species. Finally,
 56 we discuss emerging research trends in the field of rust biology, which are directly related to genomics.

57 A total of 82 Rust (Post) Genomic publications (RPGs) addressed three model rust fungi since the 58 public release of their genome sequence

59 To identify, collect, and analyze RPGs, we adapted a bibliometric pipeline we recently developed,
 60 which mostly uses iterative key word searches on the Web of Science portal (Petre et al., 2022). In
 61 total, those searches identified 82 non-redundant RPGs ([Dataset 1](#) - columns A to M). Only four RPGs
 62 addressed more than one of the three rust model species; the remaining 78 RPGs addressed specifically
 63 PST, MLP, or PGT, with 43, 20, and 15 RPGs, respectively ([Figure 1A](#)). We assigned each RPG to
 64 one of the four following categories, according to its primary finding reported and/or main approach
 65 used: i) ‘Genomics’ (22 RPGs; analysis, re-analysis, or comparison of genome sequences),
 66 ii) ‘Transcriptomics’ (14 RPGs; sRNA or mRNA profiling studies, *in laboratory* or in the field), iii)
 67 ‘Gene family analysis’ (6 RPGs; *in silico* gene family annotation and analyses), and iv) ‘functional

68 analysis' (40 RPGs; studies that functionally characterized specific molecules, mainly effector proteins
 69 – see next section). Twenty-nine RPGs reported findings that pertain to more than one category; for
 70 instance, seven RPGs from the 'Genomics' category also used transcriptomic and gene family analyses.
 71 For those 29 RPGs, we used expert reading of the manuscripts to select the most relevant primary
 72 category. RPGs from all four categories addressed MLP, PGT, and PST, indicating that all typical
 73 (post) genomic approaches are used in these three model species ([Figure 1A](#)). The number of RPGs
 74 published increased during the last decade, from 11 RPG for the 2010-2013 period to 45 for the 2018-
 75 2022 period ([Figure 1B](#)). Interestingly, the first 'functional analysis' RPGs were published only in
 76 2014, but dominate the share of RPGs published after 2018; at the opposite, RPGs from the three other
 77 categories were regularly published over the last decade. This suggests that functional investigations
 78 of rust specific proteins have emerged and then rapidly increased in number within the ten years
 79 following the sequencing of the first rust fungal genomes.

80 To better comprehend the collection of RPGs, and notably to better apprehend the publication habits
 81 of the research community, we performed a simplified citation analysis, by partially using our recently
 82 published 'HIP in' method (Petre et al., 2022). Firstly, as expected, we observed that RPGs steadily
 83 accumulate citations over time ([Figure 1C](#); [Dataset 1](#) – columns N to P). On average, RPGs receive
 84 approximately 6 citations per year (*i.e.*, annual citation rate of 6). Two of the RPGs with both the
 85 highest number of total citations and the highest annual citation rate reported reference genome
 86 sequences of MLP, PGT, and PST (Duplessis et al., 2011; Zheng et al., 2013); illustrating well the
 87 central importance of early genomic studies. Secondly, we observed that annual citations rates of the
 88 RPGs of the 'Transcriptomics' and 'Gene family analysis' negatively correlate with journal impact
 89 factors ([Figure 1D](#)). Notably, 'gene family analysis' RPGs appear in journals with modest impact
 90 factors (4,38 on average) but show high annual citations rates (10,2 on average) (Hacquard et al., 2012;
 91 Saunders et al., 2012; Sperschneider et al., 2014). Also, among the 20 RPGs with an annual citation
 92 rate superior to 8, 9 appeared in a journal with an impact factor inferior to 5. Altogether, this suggests
 93 that journal impact factors poorly predict RPGs success in terms of citation, especially for RPGs
 94 reporting gene family and transcriptome analyses. Finally, an analysis of the ten journals that published
 95 the highest number of RPGs revealed 'specialized' journals that publish only RPGs from the
 96 'genomics' or 'transcriptomics' categories (*e.g.*, BMC Genomics) or exclusively 'functional analysis'
 97 RPGs (*e.g.*, Environmental Microbiology) ([Figure 1E](#)). Interestingly, it also revealed three journals
 98 (MPMI, Frontiers in Plant Science, and PLOS ONE) that publish RPGs from all four categories and
 99 that address the three rust species; this suggests that those three journals are central in the field of rust
 100 biology, and may assist information flow between researchers tackling different research questions and
 101 objects.

102 [Pucciniales genomes facilitated the investigation of rust fungal candidate effectors](#)

103 Academic research often addresses so-called 'objects', which can notably be individual (of groups of)
 104 organisms (*e.g.*, wheat, fungi, pathogens), molecules (*e.g.*, proteins, mRNA, effectors), or structures
 105 (*e.g.*, leaves, cells, haustoria, chloroplasts). To snapshot the objects mainly addressed by RPGs, we
 106 performed a word occurrence analysis of RPG titles and abstracts, as described before ([Dataset 2](#))
 107 (Petre et al., 2022). This analysis generated a word cloud of 200 frequently-occurring words ([Figure](#)
 108 [2A](#)). Firstly, regarding the organisms, words referring to rust species or to their host (*e.g.*, 'rust',
 109 'Puccinia', 'Melampsora', 'pathogen', 'host', 'wheat', 'poplar', 'plant') dominated the word cloud,
 110 along with words referring to the two model plants *Arabidopsis thaliana* or *Nicotiana benthamiana*,
 111 which are not host to Pucciniales, but serve extensively as heterologous systems (Lorrain et al., 2018).

112 Secondly, regarding the molecules, words referring to genomes, genes, proteins, and effectors
 113 dominated the word cloud. Notably, the word effector appears in 35%, 60%, and 91% of RPG titles,
 114 abstracts, and main texts, respectively; moreover, half of the RPGs from the ‘functional analysis’
 115 category (20 out of 40) mention this word in their title. Also, 72% of the RPGs (59 out of 82)
 116 significantly addressed or discussed effectors, and in total 27 effectors were functionally characterized
 117 ([Dataset 1](#) – columns Q to T; [Figure 2B](#)). All the RPGs reporting the characterization of those 27
 118 effectors were released after 2015, 2016, or 2017 for MLP, PST, and PGT, respectively. The functional
 119 studies mostly characterized effector features pertaining to their : i) subcellular localization in plant
 120 cells (*e.g.*, nucleus, membranes, chloroplasts), ii) plant protein interactors (*e.g.*, immune receptors,
 121 candidate virulence targets), iii) ability to alter plant cell physiology (*e.g.*, alteration of sub-cellular
 122 structures and fluxes), iv) ability to trigger or suppress immunity (*e.g.*, recognition by immune
 123 receptors, elicitation or suppression of cell death or immunity), and v) tridimensional structure (*e.g.*,
 124 cystine fold) ([Figure 2B](#)). Altogether, this shows that the most recent RPGs predominantly focus on
 125 effector functional characterization, suggesting that genomes availability directly facilitated those
 126 functional studies.

127 Finally, regarding the structures, words referring to cellular structures central for the infection process
 128 (*e.g.* cells, haustoria) dominated the word cloud ([Figure 2A](#)). That was expected as haustoria are key
 129 infection structures that invaginate host cells, which have been, and still are, highly investigated in
 130 plant pathology (Hahn and Mendgen, 1997; Garnica et al., 2014; Bozkurt and Kamoun, 2020).
 131 Interestingly, the word chloroplast also appears frequently, as eight RPGs collectively reported eleven
 132 candidate effector proteins that target chloroplasts (Petre et al., 2015, 2016a, 2016b; Germain et al.,
 133 2018; Xu et al., 2019; Andac et al., 2020; Ozketen et al., 2020; Jaswal et al., 2021) ([Dataset 1](#) – columns
 134 U to W). Chloroplasts emerge as important actors of plant immunity, and the list of pathogen effectors
 135 that target chloroplast rapidly expands (Kretschmer et al., 2019; Gao et al., 2020; Littlejohn et al., 2021;
 136 Savage et al., 2021). As obligate biotrophs that intimately associate with their hosts, rust fungi probably
 137 need to deeply manipulate chloroplast functions; future studies may reveal how effector proteins assist
 138 such manipulation.

139 [Progresses made in other rust species](#)

140 Early rust genomics achievements assisted the sequencing of the genomes or of the transcriptome of
 141 other rust species, opening avenues for comparative genomics or comparative transcriptomic studies
 142 (Duplessis et al., 2013; Nemri et al., 2014; Pendleton et al., 2014; Cuomo et al., 2017; Tao et al., 2017,
 143 2019; Chen et al., 2019). Indeed, the genomes of nearly a dozen of Pucciniales species are now publicly
 144 available, and molecular resources have been compiled and made available for the community
 145 (Figuroa et al., 2020; Adams et al., 2021). In addition, progresses made with the different generations
 146 of sequencing technologies have facilitated ambitious rust sequencing projects. Indeed, the large size
 147 and the high repeat content of some rust fungal genomes has precluded their sequencing and assembly
 148 (Carvalho et al., 2014; Loehrer et al., 2014). Only recently, sequencing and assembly of larger rust
 149 genomes (of hundreds of Megabases to over a Gigabase) was made possible by including high amount
 150 of long molecule sequencing, for instance by using the PacBio HiFi technology (Wu et al., 2020;
 151 Tobias et al., 2021; Henningsen et al., 2022; Liang et al., 2022). Notably, the recent genome analysis
 152 of three isolates of the Asian soybean rust fungus *Phakospora pachyrhizi* revealed extremely high
 153 (>90%) content of transposable elements and a drastic expansion of gene families related to amino acid
 154 metabolism (Gupta et al., 2022). Finally, for a handful of rust species, such as *P. pachyrhizi*, *Hemileia*
 155 *vastatrix* (the coffee rust fungus), or *Puccinia triticina* (the wheat leaf rust fungus), coordinated studies
 156 involving genomics, transcriptomics, and gene family analyses have revealed candidate effectors; some

157 of which have been already functionally investigated (Kunjeti et al., 2016; Qi et al., 2016, 2018;
158 Segovia et al., 2016; Maia et al., 2017).

159 Towards fully phased genomes and a better understanding of Pucciniales-specific genes

160 Overall, considering the complexity of rust fungal genomes, it took about 10 years to reach a point to
161 which haplophased genome sequencing from dikaryotic spores was possible with all chromosomes
162 assembled telomere to telomere. Improvement of long read sequencing technologies such as PacBio or
163 Nanopore and emergence of methods allowing chromosome conformation capture such as Hi-C were
164 pivotal to overcome all assembly troubles related to the highly repeated profile of large rust genomes
165 (Duan et al., 2022). Despite the different pipelines used by different consortia to annotate first-
166 generation rust genomes and the heterogeneity they may have generated, they have allowed robust
167 comparative or population genomics studies (Aime et al. 2017; Lorrain et al. 2019). With more
168 systematic efforts to reach complete haplophased rust genomes, the community will be soon able to
169 draw deeper and even more robust comparative analyses to delineate evolution of gene or transposable
170 element families. Another remarkable feature of rust fungi genomes is the amount of genes of unknown
171 function, which are restricted to the order Pucciniales (Aime et al., 2017). Although a fraction of these
172 genes may be wrongly predicted, they might reflect the paucity of knowledge of the rust biology and
173 of their particularly complex life cycles (Duplessis et al., 2021). A particularly appealing and
174 challenging goal in the future of rust biology research will be to assign functions to these genes. The
175 past decade has been marked by the emergence of machine-learning driven prediction tools to identify
176 effectors or protein sub-cellular localization, such as EffectorP, Localizer, or ApoplastP (Sperschneider
177 et al., 2017a, 2017b; Sperschneider and Dodds, 2022). Improvement of such bioinformatic-assisted
178 methods will be instrumental to decipher the largely unexplored genomic landscape of the Pucciniales.

179

180 Figures captions

181 **Figure 1. Overview of the 82 Rust (Post) Genomic publications (RPGs) analyzed in this study.**

182 **(A)** Bar chart indicating the number of RPGs that primarily report genome analyses (*i.e.*, genomics;
183 brown), transcript profiling analyses (*i.e.*, transcriptomics; yellow), gene family analyses (blue), or
184 functional analyses (green), and which address *Puccinia graminis* f. sp. *tritici* (PGT), *Melampsora*
185 *larici-populina* (MLP), or *Puccinia striiformis* f. sp. *tritici* (PST). Numbers above each bar indicate the
186 total number of RPGs for each species. The four RPGs that address a combination of PGT, MLP, and
187 PST are not shown. **(B)** Bar chart indicating the evolution of the number of RPGs that pertain to
188 genomics, transcriptomics, gene family, or functional analyses throughout three time periods: 2010-
189 2013, 2014-2017, and 2018-2021; numbers and color code as in (A). The asterisks highlight the
190 ‘functional analyses’ stacks that varied from 0 RPG in 2010-2013 to 15 and 24 RPGs for the periods
191 2014-2017 and 2018-2022, respectively. **(C)** Scatterplot displaying the number of total citations of
192 individual RPGs according to their year of publication. The total number of citations was extracted
193 from the Web of Science portal in March 2022. Color code as in (A). Thirteen RPGs published in 2021
194 or 2022, and which have not yet been cited, are not shown on the graph. Acronyms indicate the three
195 RPGs reporting reference genomes in the early 2010s. **(D)** Scatterplot displaying the annual citation
196 rate of individual RPGs according to the value of the 2020 impact factor of the journal in which they
197 were published. Linear trendlines (lines) and average citation rate and impact factor values (circles)
198 are indicated for each of the four categories of RPG. Same color code as in (A). **(E)** Horizontal bar
199 chart indicating the ten journals that published the most RPGs. Stacks indicate the category (*i.e.*,
200 primary focus) of the RPGs, with the same color code as in (A). Asterisks indicate the three journals

201 that published the four types of studies. Noteworthy, 8 out of the 10 journals adopt a gold open-access
202 model. The raw data used to build this figure are available in [Dataset 1](#) (columns A to P).

203 [Figure 2. Pucciniales genomes facilitated the functional characterization of effector proteins](#)

204 **(A)** (upper panel) Word cloud displaying the most frequently appearing words referring to group of -
205 or individual - molecules, organisms, and structures. The word cloud was built by using a filtered text
206 file comprising RPGs title and abstract deprived from words not explicitly referring to molecules,
207 organisms, or structures. The word cloud displays 200 words; the size of the words positively correlates
208 with their frequency in the text file. Word colors are meaningless; they simply assist reader visual word
209 discrimination. (lower panel) Subordinate word clouds highlighting the words referring to molecules
210 (leaf-hand side) or structures (right-hand side). Grey ovals emphasize words discussed in the main text.
211 **(B)** List of effector proteins collectively investigated by the RPGs, grouped according to their rust
212 fungal species, and sorted according to the date of publication of the corresponding RPG reporting
213 their characterization. MLP: *Melampsora larici-populina*; PGT: *Puccinia graminis* f. sp. *tritici*; PST:
214 *Puccinia striiformis* f. sp. *tritici*. The asterisk indicates the non-proteinaceous, small RNA effector Pst-
215 milR1. A short summary accompanies each effector, to inform on the main findings obtained by the
216 functional studies. The raw data used to build this figure are available in [Dataset 1](#) (columns Q to W)
217 and [Dataset 2](#).

218 Conflict of Interest

219 The authors declare that the research was conducted in the absence of any commercial or financial
220 relationships that could be construed as a potential conflict of interest.

221 Author Contributions

222 Conception & design of the study (BP, SD); data acquisition, analysis, and interpretation (BP);
223 manuscript drafting (BP & SD); manuscript revision and editing (BP, SD).

224 Funding

225 The authors are supported by grants overseen by the French PIA programmes Lab of Excellence
226 ARBRE (ANR-11-LABX-0002-01), by the Pôle Scientifique A2F of the Université de Lorraine, and
227 by the Région Grand Est (France).

228 Acknowledgments

229 The authors thank members of the rust biology community, members of the UMR IAM, E. Morin,
230 J. Lintz, G. Dubrulle, G. Gygax, E. Cawston, M. Elz, A. Coince, and R. Jackson.

231 Supplementary Material

232 [Dataset 1. Dataset including RPGs and their metadata.](#)

233 [Dataset 2. filtered text file of RPGs title and abstract.](#)

234 Data Availability Statement

235 The datasets generated and analyzed in this study can be found in the supplementary material
236 accompanying this manuscript.

237 References

238 Adams, T. M., Olsson, T. S. G., Ramírez-González, R. H., Bryant, R., Bryson, R., Campos, P. E., et
239 al. (2021). Rust expression browser: an open source database for simultaneous analysis of host
240 and pathogen gene expression profiles with expVIP. *BMC Genomics* 22, 166. doi:
241 10.1186/s12864-021-07488-3.

242 Aime, M. C., and McTaggart, A. R. (2021). A higher-rank classification for rust fungi, with notes on
243 genera. *Fungal Syst. Evol.* 7, 21–47. doi: 10.3114/fuse.2021.07.02.

244 Aime, M. C., McTaggart, A. R., Mondo, S. J., and Duplessis, S. (2017). Phylogenetics and
245 Phylogenomics of Rust Fungi. *Fungal Phylogenetics Phylogenomics* 100, 267–307. doi:
246 10.1016/bs.adgen.2017.09.011.

247 Andac, A., Ozketen, A. C., Dagvadorj, B., and Akkaya, M. S. (2020). An effector of *Puccinia*
248 *striiformis* f. sp. *tritici* targets chloroplasts with a novel and robust targeting signal. *Eur. J. Plant*
249 *Pathol.* doi: 10.1007/s10658-020-02033-6.

250 Bozkurt, T. O., and Kamoun, S. (2020). The plant-pathogen haustorial interface at a glance. *J. Cell Sci.*
251 133, jcs237958. doi: 10.1242/jcs.237958.

252 Cantu, D., Govindarajulu, M., Kozik, A., Wang, M., Chen, X., Kojima, K. K., et al. (2011). Next
253 Generation Sequencing Provides Rapid Access to the Genome of *Puccinia striiformis* f. sp
254 *tritici*, the Causal Agent of Wheat Stripe Rust. *Plos One* 6, e24230. doi:
255 10.1371/journal.pone.0024230.

256 Carvalho, G. M. A., Carvalho, C. R., Barreto, R. W., and Evans, H. C. (2014). Coffee rust genome
257 measured using flow cytometry: does size matter? *Plant Pathol.* 63, 1022–1026. doi:
258 10.1111/ppa.12175.

259 Chen, J., Wu, J., Zhang, P., Dong, C., Upadhyaya, N. M., Zhou, Q., et al. (2019). De Novo Genome
260 Assembly and Comparative Genomics of the Barley Leaf Rust Pathogen *Puccinia hordei*
261 Identifies Candidates for Three Avirulence Genes. *G3-Genes Genomes Genet.* 9, 3263–3271.
262 doi: 10.1534/g3.119.400450.

263 Cuomo, C. A., Bakkeren, G., Khalil, H. B., Panwar, V., Joly, D., Linning, R., et al. (2017). Comparative
264 Analysis Highlights Variable Genome Content of Wheat Rusts and Divergence of the Mating
265 Loci. *G3-Genes Genomes Genet.* 7, 361–376. doi: 10.1534/g3.116.032797.

266 Duan, H., Jones, A. W., Hewitt, T., Mackenzie, A., Hu, Y., Sharp, A., et al. (2022). Physical separation
267 of haplotypes in dikaryons allows benchmarking of phasing accuracy in Nanopore and HiFi
268 assemblies with Hi-C data. *Genome Biol.* 23, 84. doi: 10.1186/s13059-022-02658-2.

269 Duplessis, S., Bakkeren, G., and Hamelin, R. (2014). “Advancing Knowledge on Biology of Rust
270 Fungi Through Genomics,” in *Fungi*, ed. F. M. Martin, 173–209. doi: 10.1016/B978-0-12-
271 397940-7.00006-9.

- 272 Duplessis, S., Cuomo, C. A., Lin, Y.-C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., et al. (2011).
 273 Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc. Natl. Acad.*
 274 *Sci. U. S. A.* 108, 9166–9171. doi: 10.1073/pnas.1019315108.
- 275 Duplessis, S., Lorrain, C., Petre, B., Figueroa, M., Dodds, P. N., and Aime, M. C. (2021). Host
 276 Adaptation and Virulence in Heteroecious Rust Fungi. *Annu. Rev. Phytopathol.* 59, 403–422.
 277 doi: 10.1146/annurev-phyto-020620-121149.
- 278 Duplessis, S., Spanu, P. D., and Schirawski, J. (2013). “Biotrophic Fungi (Powdery Mildews, Rusts,
 279 and Smuts),” in *The Ecological Genomics of Fungi*, ed. F. Martin (Hoboken, NJ: John Wiley
 280 & Sons, Inc), 149–168. doi: 10.1002/9781118735893.ch7.
- 281 Figueroa, M., Dodds, P. N., and Henningsen, E. C. (2020). Evolution of virulence in rust fungi -
 282 multiple solutions to one problem. *Curr. Opin. Plant Biol.* 56, 20–27. doi:
 283 10.1016/j.pbi.2020.02.007.
- 284 Figueroa, M., Upadhyaya, N. M., Sperschneider, J., Park, R. F., Szabo, L. J., Steffenson, B., et al.
 285 (2016). Changing the Game: Using Integrative Genomics to Probe Virulence Mechanisms of
 286 the Stem Rust Pathogen *Puccinia graminis* f. sp. *tritici*. *Front. Plant Sci.* 7, 205. doi:
 287 10.3389/fpls.2016.00205.
- 288 Gao, C., Xu, H., Huang, J., Sun, B., Zhang, F., Savage, Z., et al. (2020). Pathogen manipulation of
 289 chloroplast function triggers a light-dependent immune recognition. *Proc. Natl. Acad. Sci.* 117,
 290 9613–9620. doi: 10.1073/pnas.2002759117.
- 291 Garnica, D. P., Nemri, A., Upadhyaya, N. M., Rathjen, J. P., and Dodds, P. N. (2014). The Ins and
 292 Outs of Rust Haustoria. *Plos Pathog.* 10, e1004329. doi: 10.1371/journal.ppat.1004329.
- 293 Germain, H., Joly, D. L., Mireault, C., Plourde, M. B., Letanneur, C., Stewart, D., et al. (2018).
 294 Infection assays in *Arabidopsis* reveal candidate effectors from the poplar rust fungus that
 295 promote susceptibility to bacteria and oomycete pathogens: MLP effectors expressed in
 296 *Arabidopsis* affect susceptibility. *Mol. Plant Pathol.* 19, 191–200. doi: 10.1111/mpp.12514.
- 297 Gupta, Y. K., Marcelino-Guimarães, F. C., Lorrain, C., Farmer, A., Haridas, S., Ferreira, E. G. C., et
 298 al. (2022). The soybean rust pathogen *Phakopsora pachyrhizi* displays transposable element
 299 proliferation that correlates with broad host-range adaptation on legumes. *BioRxiv*. doi:
 300 10.1101/2022.06.13.495685.
- 301 Hacquard, S., Joly, D. L., Lin, Y.-C., Tisserant, E., Feau, N., Delaruelle, C., et al. (2012). A
 302 Comprehensive Analysis of Genes Encoding Small Secreted Proteins Identifies Candidate
 303 Effectors in *Melampsora larici-populina* (Poplar Leaf Rust). *Mol. Plant. Microbe Interact.* 25,
 304 279–293. doi: 10.1094/MPMI-09-11-0238.
- 305 Hahn, M., and Mendgen, K. (1997). Characterization of in planta induced rust genes isolated from a
 306 haustorium-specific cDNA library. *Mol. Plant. Microbe Interact.* 10, 427–437. doi:
 307 10.1094/MPMI.1997.10.4.427.
- 308 Henningsen, E. C., Hewitt, T., Dugyala, S., Nazareno, E. S., Gilbert, E., Li, F., et al. (2022). A
 309 chromosome-level, fully phased genome assembly of the oat crown rust fungus *Puccinia*

- 310 coronata f. sp. avenae: a resource to enable comparative genomics in the cereal rusts. *G3*
311 *GenesGenomesGenetics*, 10. doi: <https://doi.org/10.1093/g3journal/jkac149>.
- 312 Jaswal, R., Rajarammohan, S., Dubey, H., Kiran, K., Rawal, H., Sonah, H., et al. (2021). A kiwellin
313 protein-like fold containing rust effector protein localizes to chloroplast and suppress cell death
314 in plants. *BioRxiv*. doi: 10.1101/2021.08.20.456821.
- 315 Kretschmer, M., Damoo, D., Djamei, A., and Kronstad, J. (2019). Chloroplasts and Plant Immunity:
316 Where Are the Fungal Effectors? *Pathogens* 9, 19. doi: 10.3390/pathogens9010019.
- 317 Kunjeti, S. G., Iyer, G., Johnson, E., Li, E., Broglie, K. E., Rauscher, G., et al. (2016). Identification of
318 Phakopsora pachyrhizi Candidate Effectors with Virulence Activity in a Distantly Related
319 Pathosystem. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00269.
- 320 Liang, J., Li, Y., Dodds, P. N., Figueroa, M., Sperschneider, J., Han, S., et al. (2022). Haplotype-phased
321 and chromosome-level genome assembly of *Puccinia polysora*, a giga-scale fungal pathogen
322 causing southern corn rust. *Genomics* doi: 10.1101/2022.05.18.492400.
- 323 Littlejohn, G. R., Breen, S., Smirnov, N., and Grant, M. (2021). Chloroplast immunity illuminated.
324 *New Phytol.* 229, 3088–3107. doi: 10.1111/nph.17076.
- 325 Loehrer, M., Botterweck, J., Jahnke, J., Mahlmann, D. M., Gaetgens, J., Oldiges, M., et al. (2014).
326 In vivo assessment by Mach-Zehnder double-beam interferometry of the invasive force exerted
327 by the Asian soybean rust fungus (*Phakopsora pachyrhizi*). *New Phytol.* 203, 620–631. doi:
328 10.1111/nph.12784.
- 329 Lorrain, C., dos Santos, K. C. G., Germain, H., Hecker, A., and Duplessis, S. (2019). Advances in
330 understanding obligate biotrophy in rust fungi. *New Phytol.* 222, 1190–1206. doi:
331 10.1111/nph.15641.
- 332 Lorrain, C., Petre, B., and Duplessis, S. (2018). Show me the way: rust effector targets in heterologous
333 plant systems. *Curr. Opin. Microbiol.* 46, 19–25. doi: 10.1016/j.mib.2018.01.016.
- 334 Maia, T., Badel, J. L., Marin-Ramirez, G., Rocha, C. de M., Fernandes, M. B., Silva, J. C. F., et al.
335 (2017). The *Hemileia vastatrix* effector HvEC-016 suppresses bacterial blight symptoms in
336 coffee genotypes with the SH1 rust resistance gene. *New Phytol.* 213, 1315–1329. doi:
337 10.1111/nph.14334.
- 338 Moscou, M. J., and van Esse, H. P. (2017). The quest for durable resistance. *Science* 358, 1541–1542.
339 doi: 10.1126/science.aar4797.
- 340 Nemri, A., Saunders, D. G. O., Anderson, C., Upadhyaya, N. M., Win, J., Lawrence, G. J., et al. (2014).
341 The genome sequence and effector complement of the flax rust pathogen *Melampsora lini*.
342 *Front. Plant Sci.* 5, 98. doi: 10.3389/fpls.2014.00098.
- 343 Ozketen, A. C., Andac-Ozketen, A., Dagvadorj, B., Demiralay, B., and Akkaya, M. S. (2020). In-depth
344 secretome analysis of *Puccinia striiformis* f. sp. *tritici* in infected wheat uncovers effector
345 functions. *Biosci. Rep.* 40, BSR20201188. doi: 10.1042/BSR20201188.

- 346 Pendleton, A. L., Smith, K. E., Feau, N., Martin, F. M., Grigoriev, I. V., Hamelin, R., et al. (2014).
347 Duplications and losses in gene families of rust pathogens highlight putative effectors. *Front.*
348 *Plant Sci.* 5. doi: 10.3389/fpls.2014.00299.
- 349 Petre, B., Lorrain, C., Saunders, D. G. O., Win, J., Sklenar, J., Duplessis, S., et al. (2016a). Rust fungal
350 effectors mimic host transit peptides to translocate into chloroplasts: Effectors use molecular
351 mimicry to target chloroplasts. *Cell. Microbiol.* 18, 453–465. doi: 10.1111/cmi.12530.
- 352 Petre, B., Louet, C., Lintz, J., and Duplessis, S. (2022). 2000–2019: twenty years of highly influential
353 publications in molecular plant immunity. *Mol. Plant. Microbe Interact.* doi: 10.1094/MPMI-
354 05-22-0112-CR.
- 355 Petre, B., Saunders, D. G. O., Sklenar, J., Lorrain, C., Krasileva, K. V., Win, J., et al. (2016b).
356 Heterologous Expression Screens in *Nicotiana benthamiana* Identify a Candidate Effector of
357 the Wheat Yellow Rust Pathogen that Associates with Processing Bodies. *PLOS ONE* 11,
358 e0149035. doi: 10.1371/journal.pone.0149035.
- 359 Petre, B., Saunders, D. G. O., Sklenar, J., Lorrain, C., Win, J., Duplessis, S., et al. (2015). Candidate
360 Effector Proteins of the Rust Pathogen *Melampsora larici-populina* Target Diverse Plant Cell
361 Compartments. *Mol. Plant-Microbe Interactions*® 28, 689–700. doi: 10.1094/MPMI-01-15-
362 0003-R.
- 363 Qi, M., Grayczyk, J. P., Seitz, J. M., Lee, Y., Link, T. I., Choi, D., et al. (2018). Suppression or
364 Activation of Immune Responses by Predicted Secreted Proteins of the Soybean Rust Pathogen
365 *Phakopsora pachyrhizi*. *Mol. Plant. Microbe Interact.* 31, 163–174. doi: 10.1094/MPMI-07-
366 17-0173-FI.
- 367 Qi, M., Link, T. I., Mueller, M., Hirschburger, D., Pudake, R. N., Pedley, K. F., et al. (2016). A Small
368 Cysteine-Rich Protein from the Asian Soybean Rust Fungus, *Phakopsora pachyrhizi*,
369 Suppresses Plant Immunity. *Plos Pathog.* 12, e1005827. doi: 10.1371/journal.ppat.1005827.
- 370 Saunders, D. G. O., Win, J., Cano, L. M., Szabo, L. J., Kamoun, S., and Raffaele, S. (2012). Using
371 Hierarchical Clustering of Secreted Protein Families to Classify and Rank Candidate Effectors
372 of Rust Fungi. *Plos One* 7, e29847. doi: 10.1371/journal.pone.0029847.
- 373 Savage, Z., Duggan, C., Toufexi, A., Pandey, P., Liang, Y., Segretin, M. E., et al. (2021). Chloroplasts
374 alter their morphology and accumulate at the pathogen interface during infection by
375 *Phytophthora infestans*. *Plant J.* 107, 1771–1787. doi: 10.1111/tpj.15416.
- 376 Segovia, V., Bruce, M., Shoup Rupp, J. L., Huang, L., Bakkeren, G., Trick, H. N., et al. (2016). Two
377 small secreted proteins from *Puccinia triticina* induce reduction of β -glucuronidase transient
378 expression in wheat isolines containing *Lr9*, *Lr24* and *Lr26*. *Can. J. Plant Pathol.* 38, 91–102.
379 doi: 10.1080/07060661.2016.1150884.
- 380 Sperschneider, J., Catanzariti, A.-M., DeBoer, K., Petre, B., Gardiner, D. M., Singh, K. B., et al.
381 (2017a). LOCALIZER: subcellular localization prediction of both plant and effector proteins
382 in the plant cell. *Sci. Rep.* 7, 44598. doi: 10.1038/srep44598.

- 383 Sperschneider, J., and Dodds, P. N. (2022). EffectorP 3.0: Prediction of Apoplastic and Cytoplasmic
384 Effectors in Fungi and Oomycetes. *Mol. Plant-Microbe Interactions*® 35, 146–156. doi:
385 10.1094/MPMI-08-21-0201-R.
- 386 Sperschneider, J., Dodds, P. N., Taylor, J. M., and Duplessis, S. (2017b). “Computational Methods for
387 Predicting Effectors in Rust Pathogens,” in *Wheat Rust Diseases: Methods and Protocols*, ed.
388 S. Periyannan, 73–83. doi: 10.1007/978-1-4939-7249-4_7.
- 389 Sperschneider, J., Ying, H., Dodds, P. N., Gardiner, D. M., Upadhyaya, N. M., Singh, K. B., et al.
390 (2014). Diversifying selection in the wheat stem rust fungus acts predominantly on pathogen-
391 associated gene families and reveals candidate effectors. *Front. Plant Sci.* 5. doi:
392 10.3389/fpls.2014.00372.
- 393 Tao, S.-Q., Cao, B., Morin, E., Liang, Y.-M., and Duplessis, S. (2019). Comparative transcriptomics
394 of *Gymnosporangium* spp. teliospores reveals a conserved genetic program at this specific stage
395 of the rust fungal life cycle. *Bmc Genomics* 20, 723. doi: 10.1186/s12864-019-6099-x.
- 396 Tao, S.-Q., Cao, B., Tian, C.-M., and Liang, Y.-M. (2017). Comparative transcriptome analysis and
397 identification of candidate effectors in two related rust species (*Gymnosporangium yamadae*
398 and *Gymnosporangium asiaticum*). *Bmc Genomics* 18, 651. doi: 10.1186/s12864-017-4059-x.
- 399 Tobias, P. A., Schwessinger, B., Deng, C. H., Wu, C., Dong, C., Sperschneider, J., et al. (2021).
400 *Austropuccinia psidii*, causing myrtle rust, has a gigabase-sized genome shaped by
401 transposable elements. *G3 GenesGenomesGenetics* 11, jkaa015. doi:
402 10.1093/g3journal/jkaa015.
- 403 Wu, J. Q., Dong, C., Song, L., and Park, R. F. (2020). Long-Read-Basedde novoGenome Assembly
404 and Comparative Genomics of the Wheat Leaf Rust PathogenPuccinia triticinaIdentifies
405 Candidates for Three Avirulence Genes. *Front. Genet.* 11, 521. doi: 10.3389/fgene.2020.00521.
- 406 Xu, Q., Tang, C., Wang, X., Sun, S., Zhao, J., Kang, Z., et al. (2019). An effector protein of the wheat
407 stripe rust fungus targets chloroplasts and suppresses chloroplast function. *Nat. Commun.* 10,
408 5571. doi: 10.1038/s41467-019-13487-6.
- 409 Zheng, W., Huang, L., Huang, J., Wang, X., Chen, X., Zhao, J., et al. (2013). High genome
410 heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. *Nat.*
411 *Commun.* 4, 2673. doi: 10.1038/ncomms3673.

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