

1 **Maintenance of variation in virulence and reproduction in populations of an**
2 **agricultural plant pathogen**

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4 Anik Dutta¹, Daniel Croll², Bruce A. McDonald¹, and Luke G. Barrett³, *

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6 ¹Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

7 ²Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel,

8 Neuchâtel, Switzerland

9 ³CSIRO Agriculture and Food, Canberra, Australian Capital Territory, Australia

10

11 ***Corresponding author:** Luke G. Barrett; E-mail: luke.barrett@csiro.au

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29 **Abstract**

30 Genetic diversity within pathogen populations is critically important for predicting pathogen
31 evolution, disease outcomes and prevalence. However, we lack a good understanding of the
32 processes maintaining genetic variation and constraints on pathogen life-history
33 evolution. Here, we analyzed interactions between 12 wheat host genotypes and 145 strains of
34 *Zymoseptoria tritici* from five global populations to investigate the evolution and maintenance
35 of variation in pathogen virulence and reproduction. We found a strong positive correlation
36 between virulence and reproduction, with substantial variation in both traits maintained within
37 each pathogen population. On average, highly virulent isolates exhibited higher fecundity,
38 which might increase transmission potential in agricultural fields planted to homogeneous
39 hosts at a high density. We further showed that pathogen strains with a narrow host range (i.e.
40 specialists) for fecundity were on average less virulent, and those with a broader host range
41 (i.e. generalists) for virulence were on average less fecund on a given specific host. These
42 trade-offs costs associated with host specialization might constrain the directional evolution of
43 virulence and fecundity. We conclude that selection favoring pathogen strains that are virulent
44 across diverse hosts, coupled with selection that maximizes fecundity on specific hosts, may
45 explain the maintenance of these pathogenicity traits within and among pathogen populations.

46

47 **Keywords:** *Zymoseptoria tritici*, wheat, virulence, reproduction, trade-off, host specialization.

48

49 **Introduction**

50 Plant pathogens typically maintain high intraspecies diversity for key pathogenic traits. These
51 include virulence (defined here as damage caused to the host), host range, and reproduction
52 (Lannou, 2012). Genetic variation underlying phenotypic trait variation (and corresponding
53 resistance traits in their hosts) can be an important determinant of disease epidemiology and
54 can have important consequences for pathogen fitness, host mortality and host reproduction.
55 For example, genetic variation within a pathogen species can facilitate rapid adaptation to
56 control strategies such as fungicide applications and deployment of host resistance (McDonald
57 and Linde, 2002; Zhan *et al.*, 2005). However, despite the importance of pathogenicity traits
58 for determining disease incidence, prevalence, and severity, we still lack a clear understanding
59 of how they evolve within populations.

60

61 Virulence in plant pathogens can be defined as the degree of damage (e.g., necrosis) and the
62 corresponding fitness reduction in the host following a pathogen infection (Sacristan and
63 Garcia-Arenal, 2008; Barrett *et al.*, 2009). Virulence is (at least in part) a direct consequence
64 of host exploitation and is therefore expected to have strong links with pathogen growth and
65 the development of transmission stages needed to infect new hosts (i.e. fecundity). Virulence
66 is thus a key component of pathogen life-history, influencing both the incidence and impact of
67 disease. Populations of plant pathogens typically harbor high levels of genetic variation for
68 both virulence and fecundity (Sacristan and Garcia-Arenal, 2008; Barrett *et al.*, 2009).
69 Furthermore, several studies have demonstrated variable expression of these traits according
70 to host and pathogen genetic background, their interaction, and environmental effects in
71 different host-pathogen systems (Pagan *et al.*, 2007; Salvaudon *et al.*, 2007; Lannou, 2012;
72 Tack *et al.*, 2012). However, despite the central importance of these traits to pathogenicity, the

73 processes underlying their evolution and the maintenance of variation within populations are
74 not well understood.

75

76 While fecundity is an important life-history trait, pathogen fitness also critically depends upon
77 the transmission of propagules to a new host (Antonovics, 2017). While increased pathogen
78 fecundity can increase potential for transmission, links between virulence, fecundity and
79 transmission are complicated by the fact that transmission also depends on the host. This
80 complexity is captured by the so called ‘trade-off’ theory, which assumes that virulence is an
81 unavoidable consequence of pathogen reproduction within the host (Lenski and May, 1994;
82 Frank, 1996), and predicts that intermediate levels of virulence will maximize transmission
83 because higher virulence may significantly reduce the life expectancy of the infected host
84 (Anderson and May, 1982; Frank, 1996; Leggett *et al.*, 2013). However, the trade-off theory
85 has largely been developed within the context of unmanaged host populations (e.g. humans,
86 wild animals etc.). Here, we examine the evolution of virulence and fecundity within
87 populations of an agricultural plant pathogen. There are some key properties specific to
88 agroecosystems that might influence expectations for the evolution of virulence. Unlike natural
89 systems, hosts are homogenous and planted at a high density (allowing frequent physical
90 contact among plants), properties that potentially select for increasingly high virulence and
91 reproduction (McDonald and Stukenbrock, 2016). In addition, farmers replace these high
92 density, homogenous resources every year, meaning that there are potentially few negative
93 consequences associated with increasing levels of host damage. The evolution of virulence in
94 agroecosystems can be further influenced by the pleiotropic effect of genes affecting fungicide
95 resistance in pathogen populations. Extensive fungicide application can impose strong
96 directional selection for resistance, which can be positively correlated with virulence (Yang *et*
97 *al.*, 2013). Thus, it might be predicted that in agricultural settings, directional selection may

98 result in uniformly high levels of virulence and fecundity (Walsh and Blows, 2009; Roff,
99 2012).

100

101 Countering this prediction are frequent reports of high levels of pathogenic diversity within
102 populations of agricultural plant pathogens (Burdon *et al.*, 2016). Trade-offs between different
103 components of fitness are often invoked to explain the maintenance of trait variation within
104 species (Stearns, 1989; Thrall *et al.*, 2005; Héraudet *et al.*, 2008). For host-pathogen
105 interactions, one common prediction is that host specialization will result in the evolution of
106 high levels of fecundity on a restricted subset of hosts (Barrett and Heil, 2012). This outcome
107 implies the existence of trade-offs between the capacity to attack multiple hosts and another
108 component of fitness. A broader host range (i.e. generalism) is expected to increase the number
109 of individual hosts available to infect and lower the risk of extinction should any one host
110 become unavailable, whereas specialization on any individual host (i.e. specialism) comes at
111 the expense of reduced performance on other possible hosts, creating an evolutionary constraint
112 (Kassen, 2002). This specialization trade-off is frequently used to explain why pathogenic traits
113 do not become fixed (i.e. the trade-off maintains trait diversity) in pathogen populations
114 (Brown, 2003). Yet, empirical evidence for costs arising from host specialization are limited
115 (Barrett and Heil, 2012).

116

117 *Z. tritici* is the causal agent of septoria tritici blotch, a major fungal disease of wheat (Dean *et*
118 *al.*, 2012; Fones and Gurr, 2015). The pathogen causes necrotic lesions upon infection and
119 produces asexual fruiting bodies called pycnidia within the lesions. It undergoes several cycles
120 of a/sexual reproduction in a growing season (Karisto *et al.*, 2018). Lesion development and
121 pycnidia formation were shown to be two different traits, which can vary according to the
122 particular genotypes involved in each host-pathogen interaction (Karisto *et al.*, 2018). In this

123 study, we used lesion development and pycnidia formation within lesions as proxies for
124 pathogen virulence and reproduction, respectively. We used a set of 12 wheat host cultivars
125 and 145 *Zymoseptoria tritici* strains to address the following questions: To what extent do key
126 pathogenic life-history traits vary within and among populations of an agricultural plant
127 pathogen? How do pathogen virulence and reproductive traits correlate? What is the impact of
128 spatial structure (i.e. hosts and pathogen populations) on variation in virulence and
129 reproduction? Is there any evidence for a trade-off between specialist and generalist strategies,
130 and if so, what other traits are involved?

131

132 **Materials and Methods**

133 *Fungal material*

134 Five genetically different pathogen populations comprising 145 fully sequenced isolates of *Z.*
135 *tritici* were used in this study. These field populations originated from single wheat fields
136 located in four countries around the world (Zhan *et al.*, 2005). The field populations Australia
137 ($n=27$), Switzerland ($n=32$), Israel ($n=30$), and USA (Oregon.R, $n=26$; Oregon.S, $n=30$) were
138 collected in 2001, 1999, 1991 and 1990, respectively. The two populations from Oregon were
139 collected on the same day from two different wheat cultivars, Madsen (Oregon.R) and Stephens
140 (Oregon.S), growing in the same field. All the other populations were sampled from single
141 cultivars. The cultivars Madsen and Stephens were partially resistant and highly susceptible to
142 STB, respectively (Cowger *et al.*, 2000). The absence of clones among these isolates and the
143 general absence of pathogen clones within and among populations beyond spatial scales of 1
144 m was confirmed by previous studies (Linde *et al.*, 2002; Zhan *et al.*, 2005). After collection,
145 several copies of each isolate were stored and maintained in anhydrous silica gel or 50%
146 glycerol at -80°C .

147 *Plant material*

148 A set of 12 different wheat hosts were used in this study. This host panel included five landraces
149 (Chinese Spring, 1011, 1204, 4391 and 5254), six commercial varieties (Drifter, Gene, Greina,
150 Runal, Titlis, Toronit) and a back-cross line (ArinaLr34). The 1011, 1204, 4391, and 5254
151 landraces were selected from a collection of 199 Swiss wheat landraces from the Swiss national
152 gene bank (www.bdn.ch). This panel was screened for resistance to STB (unpublished results)
153 using four fully sequenced *Z. tritici* isolates, namely 3D1, 3D7, 1E4 and 1A5 (Lendenmann *et*
154 *al.*, 2014; Croll *et al.*, 2013). The landraces 1011 and 4391 were highly resistant and
155 susceptible, respectively, to all four isolates. The landraces 1204 and 5254 were moderately
156 susceptible to the four isolates. The remaining hosts have diverse genetic backgrounds and
157 were previously used to identify infectivity (i.e. a/virulence) factors in *Z. tritici* (Hartmann *et*
158 *al.*, 2017; Zhong *et al.*, 2017; Meile *et al.*, 2018; Stewart *et al.*, 2018). The seeds of Gene and
159 ArinaLr34 were provided by Christopher Mundt (Oregon State University) and Simon
160 Krattinger (KAUST), respectively. The seeds of other hosts were obtained from DSP Ltd.
161 (Delley, Switzerland).

162

163 *Preparation of fungal inoculum*

164 The isolates were regenerated from long term glycerol storage by adding 40µl of concentrated
165 spore suspension into 100 ml Erlenmeyer flasks containing 50ml of yeast sucrose broth (YSB,
166 10 g/L sucrose, 10 g/L yeast extract amended with 50 µg/ml kanamycin sulfate to control other
167 microbial growth). The flasks were kept at 18°C on a continuous shaker at 120 rpm to produce
168 blastospores. Blastospores were harvested after 4-7 days of growth by filtering the liquid media
169 through two layers of sterile cheesecloth. Blastospore pellets were collected by centrifugation
170 (1575 g, 15 minutes, 4°C), washed with sterile water to eliminate any residual growth media
171 and re-suspended in sterile water for subsequent procedures. The spores were counted and
172 adjusted to a final concentration of 5×10⁶ spores/ml using KOVA counting slides (Hycor

173 Biomedical, Inc., Garden Grove, CA, USA). The spore suspension of each isolate was stored
174 at -20°C until inoculation for between 1-21 days.

175

176 *Phenotyping and data collection*

177 Due to greenhouse space limitations, the whole experiment was divided into two phases
178 consisting of a combination of 6 hosts × 145 isolates in each phase. Three seeds of each host
179 were sown in an individual square pot filled with peat substrate Jiffy GO PP7 (Jiffy Products
180 International, Moerdijk, the Netherlands). Six pots were placed on a tray in a 2×3 array. All the
181 trays were kept in a greenhouse at 22°C (day) and 18°C (night) with 70% relative humidity
182 (RH) and 16-h photoperiod. Plants were inoculated after developing a fully expanded second
183 leaf, at 14 days after sowing. The third leaf and subsequent leaves were trimmed before
184 inoculation and new leaves were trimmed until data collection to facilitate a more uniform
185 distribution of spores and better light penetration onto the inoculated leaf. Before the
186 inoculation day, the spore suspension of each isolate was thawed on ice and kept at 4°C
187 overnight. The final volume of each spore suspension used for inoculation was adjusted to 20
188 ml by adding sterile water supplemented with 0.1% of the Tween 20 surfactant. Each tray
189 containing six hosts was inoculated with an airbrush spray gun (1A Profi Handels GmbH,
190 Wiesbaden, Germany) uniformly until run-off. The spraying was done in a confined area to
191 minimize any chance of cross-contamination. The trays were covered with plastic bags after
192 inoculation to provide 100% RH and transferred into a greenhouse chamber. The entire
193 procedure was repeated three times over three consecutive weeks to generate three independent
194 biological replicates for both phases of the experiment. Spraying of all 145 isolates was
195 performed on a single day. For the landraces 1011, 5254 and 1204, 4391, only one and two
196 plants, respectively, were inoculated in each replicate due to limited seed availability.

197

198 Plastic bags were removed three days post-inoculation (dpi). Environmental conditions
199 otherwise remained identical. To facilitate comparisons among hosts, leaves were harvested
200 between 19-26 dpi because different hosts developed symptoms at different rates. All leaves
201 from each host×isolate combination were collected on the same day. Each second leaf was
202 excised and mounted on A4 paper for scanning as described previously (Karisto *et al.*, 2018).
203 Each A4 sheet containing eight inoculated leaves was scanned using a flatbed scanner
204 (CanoScan LiDE 220) for automated image analysis (AIA; Karisto *et al.*, 2018). The AIA
205 provided quantitative data on the amount of lesion area caused by the fungus and pycnidia
206 density within the lesions on each leaf. As previously described in Karisto *et al.*, (2018), lesion
207 area corresponds to host damage and was used as a proxy for virulence, whereas pycnidia
208 density is a direct measure of pathogen asexual reproduction as pycnidia produce the spores
209 that are eventually transmitted through rain splash or direct contact to neighboring plants.

210

211 *Data analysis*

212 Before analysis, all phenotypic values were log-transformed to fulfill the normality
213 assumptions of ANOVA based on the residual distribution. The log-transformed values were
214 used for all analyses. A combined analysis of variance (ANOVA) was performed to estimate
215 the effect of different factors on the two traits. The following linear model was implemented
216 using the *lm()* function from the package lme4 (Bates *et al.*, 2014) in R (R core team, 2019):

217 *Virulence/Reproduction*~*Host+Population+Isolate:Population+Host:Population+*

218 *Host:Isolate:Population+Replication+Error*

219 All factors were treated as fixed effects and isolates were nested within each population. The
220 least-square mean (LSmean) for each host×isolate combination was extracted by using the
221 function “emmeans” from the package emmeans (Lenth, 2018). Using the LSmean from each
222 host, we computed the global mean for each isolate for virulence and reproduction. Posthoc

223 multiple comparisons of LSmeans of the host and pathogen population interactions were
224 performed by using Tukey's HSD test at the $\alpha=0.05$ significance level.

225

226 We used the coefficient of variation (CV = standard deviation/mean) of LSmeans for each
227 isolate among 12 hosts as a metric for host specificity (Poissot *et al.*, 2012; Caseys *et al.*, 2019).
228 Several methods including CV have been proposed for measuring specificity at both individual
229 and species levels (Bolnic *et al.*, 2002; Poissot *et al.*, 2012). However, each of these has
230 limitations depending on the type of data. While the majority of the specificity metrics assume
231 discrete data, very few are available for continuous data. In our dataset, the use of host
232 resources was more evenly distributed. The majority of isolates readily infected the majority
233 of susceptible hosts, while the degree of virulence and the amount of reproduction varied
234 among the majority of them. Hence we favored the use of CV, which facilitates estimation of
235 the degree of specialism and generalism among the isolates using precise quantitative
236 phenotypic data.

237

238 To test for trade-offs, we performed Pearson's correlation analysis between virulence and
239 reproduction, using the overall mean across 12 hosts. Furthermore, we tested the shape of the
240 correlation curve by fitting a polynomial model (*Reproduction~poly (Virulence, 2)*). We were
241 interested in determining how the specificity of each isolate affects its mean virulence and
242 reproduction. Therefore, we performed correlations between mean virulence and the specificity
243 of reproduction as well as between mean reproduction and the specificity of virulence. All
244 analyses were performed in R-studio.

245 **Results**

246 *Determinants of quantitative variation in pathogen virulence and reproduction*

247 We obtained quantitative data from 11'019 inoculated leaves generated from the interactions
248 between 12 hosts and 145 pathogen strains using AIA to investigate how variation in pathogen
249 life-history traits are maintained. Lesion area and pycnidia density within the lesion area were
250 used as proxies of virulence and reproduction, respectively. Isolates displayed a wide range of
251 phenotypic responses, exhibiting a quantitative distribution in virulence and reproduction on
252 each host (Supplemental figs. 1A and 1B). Using the non-transformed data, virulence ranged
253 from 0 to 100% with an overall mean of $61.4 \pm 0.4\%$. The host 1011 showed the highest
254 resistance on average, followed by Gene and Toronit (Supplemental Table 1). Mean
255 reproduction across 12 hosts ranged from 0 to 671 pycnidia per cm² of lesion with an overall
256 mean of 17.8 ± 0.4 . The isolates were on average highly virulent and fecund on the most
257 susceptible host 4391.

258

259 We applied a linear model to test which factors contributed to the observed quantitative
260 variation in virulence and reproduction. There was significant variation ($P < 2e-16$) among the
261 hosts, populations, and isolates for both the traits (Table 1). Host identity strongly influenced
262 the observed variation in reproduction ($F = 835.53$, $P < 2e-16$) and virulence ($F = 232.47$, $P <$
263 $2e-16$). We obtained a highly significant interaction effect between hosts and isolates nested
264 within each population for virulence ($F = 2.11$, $P < 2e-16$) and reproduction ($F = 2.68$, $P < 2e-$
265 16). This significant interaction effect indicates the occurrence of host specificity among the
266 isolates for both traits. Multiple comparisons among the populations on each host revealed high
267 variability and changes in ranking among the populations for both traits (Figs. 1A and 1B). The
268 two populations from Oregon had the highest overall mean virulence. The Israeli population
269 exhibited the highest reproduction, followed by Oregon.R and Switzerland.

270

271 **Table 1.** Analysis of Variance (ANOVA) showing the effects of hosts, populations, isolates
 272 and the respective interactions on virulence (amount of necrotic lesion area) and
 273 reproduction (pycnidia density within lesions) among 145 *Zymoseptoria tritici*
 274 isolates from five populations across 12 hosts.

Sources of variation	Virulence				Reproduction		
	DF	SS	F-value	P-value	SS	F-value	P-value
Population	4	77.36	105.42	<2e-16***	98.19	168.16	<2e-16***
Population:Isolate	140	329.50	12.83	<2e-16***	275.80	13.49	<2e-16***
Host	11	469.08	232.47	<2e-16***	1341.63	835.53	<2e-16***
Host:Population	44	171.96	21.30	<2e-16***	387.13	60.27	<2e-16***
Host:Population:Isolate	1540	596.08	2.11	<2e-16***	603.40	2.68	<2e-16***
Replication	2	8.07	22.00	6.7e-16***	4.16	14.23	6.7e-16***
Error	9277	1701.74			1354.21		

275 *** indicates significance level at 0.01%; DF= degrees of freedom; SS=Sum of square

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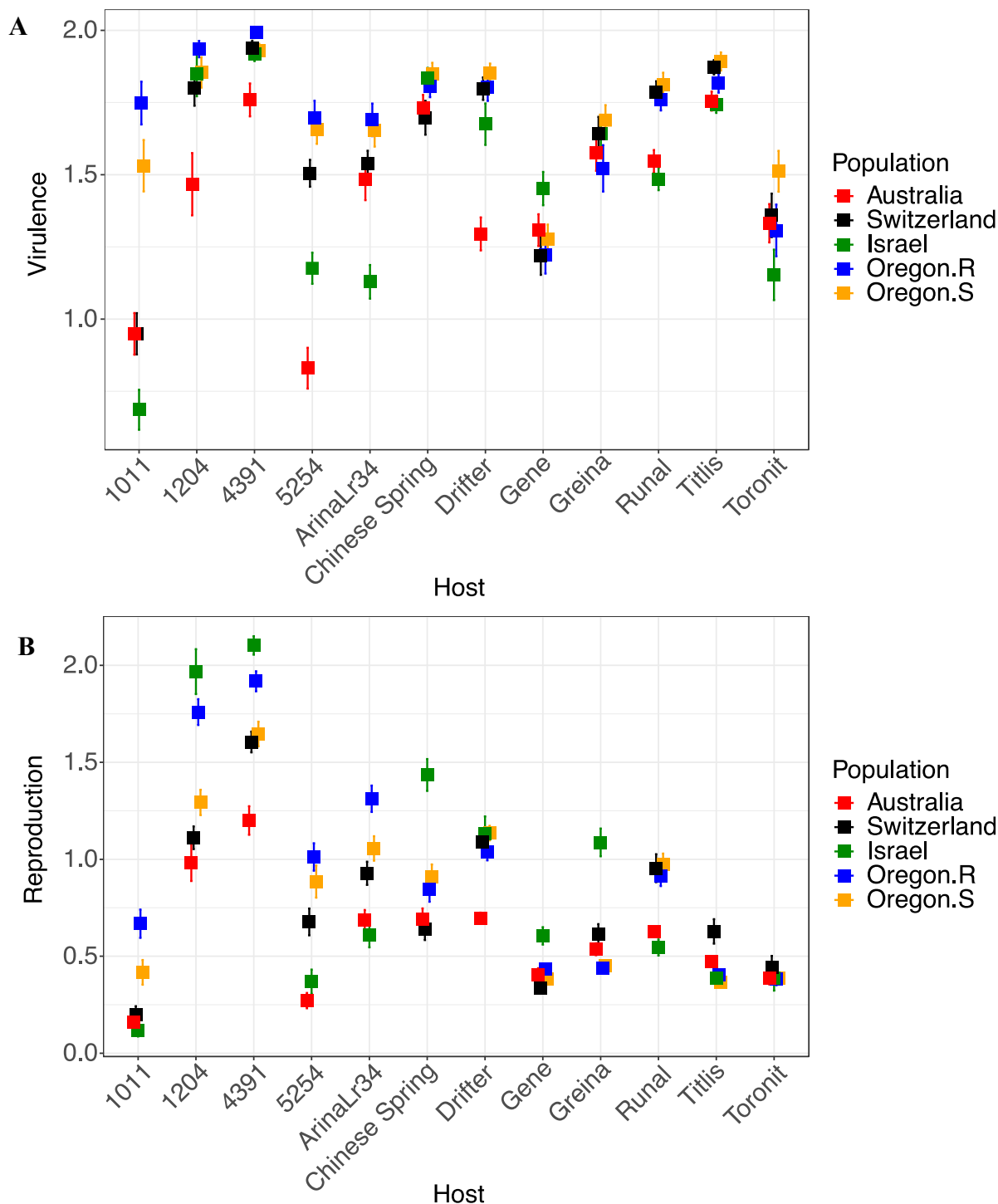
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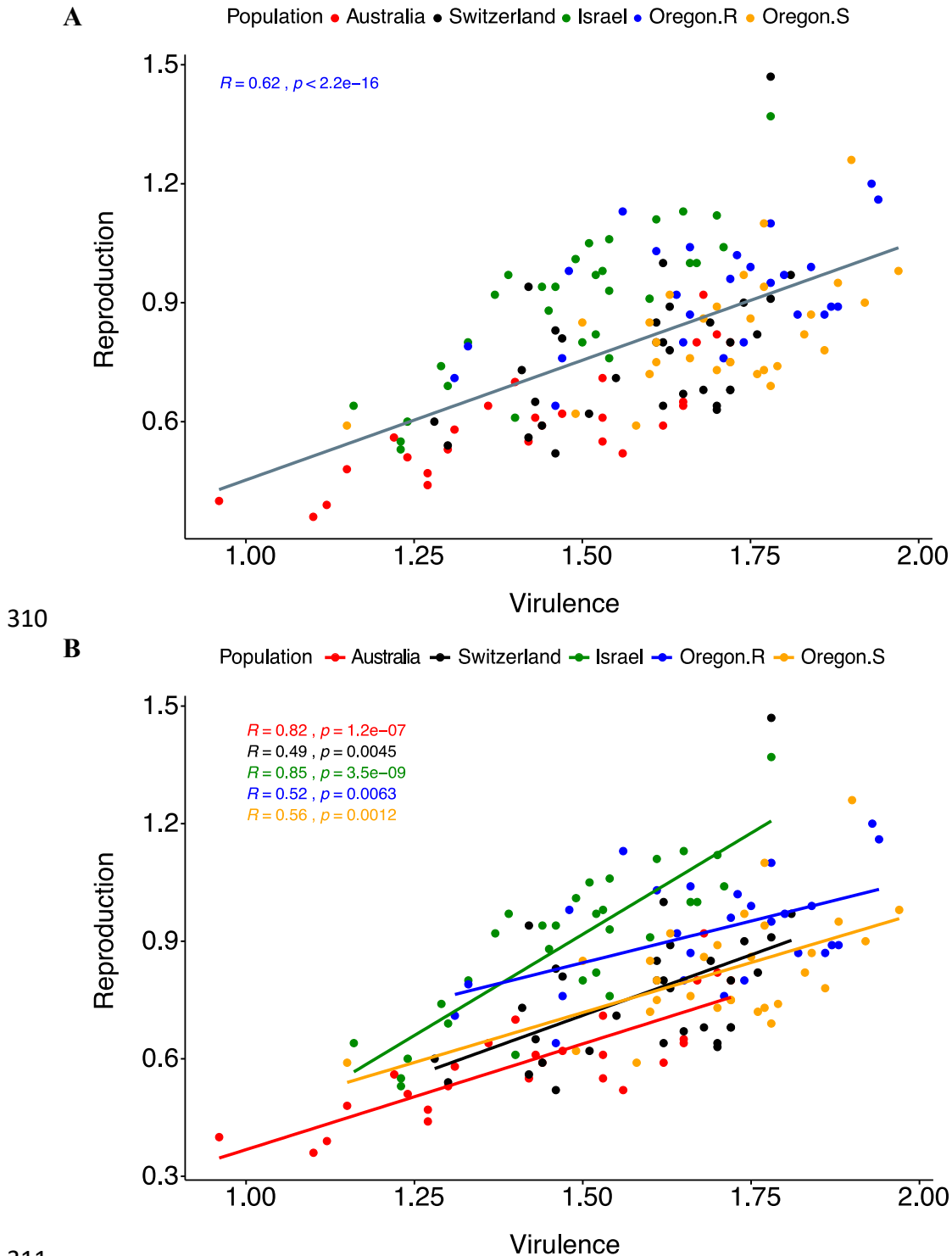


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291 **Figure 1.** Multiple comparisons for (A) virulence (amount of necrotic lesion area) and (B)
292 reproduction (pycnidia density within lesions) among the five *Zymoseptoria tritici*
293 populations on 12 wheat hosts. Data were log-transformed.
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297 *Absence of a trade-off between pathogen virulence and reproduction*

298 We performed Pearson's correlation between virulence and reproduction to determine whether
299 or not there is a trade-off between these traits. The overall mean values of each isolate across
300 12 hosts were used in this analysis. Overall, we detected a significant positive correlation ($r =$
301 0.62 , $P < 2.2e-16$; Fig. 2A) between the two traits. This indicates that highly virulent isolates
302 also had high levels of fecundity. However, the polynomial regression did not show evidence
303 for any saturating point (results not shown) on the curve, indicating an increasing trend for both
304 traits. The positive correlation was consistent within each population, although the strength of
305 the correlation within each population varied considerably, with the ISR population showing
306 the highest correlation coefficient ($r = 0.49$ to 0.85 , $P = 0.0045$ to $3.5e-09$; Fig. 2B). The
307 variation in the correlation indicated that individual isolates might differ in their strategy to
308 exploit certain hosts.

309



311
312 **Figure 2.** Correlation between virulence (amount of necrotic lesion area) and reproduction
313 (pycnidia density within lesions; A) overall and (B) within each population, among
314 145 *Zymoseptoria tritici* isolates from five populations. Each point represents the
315 overall.

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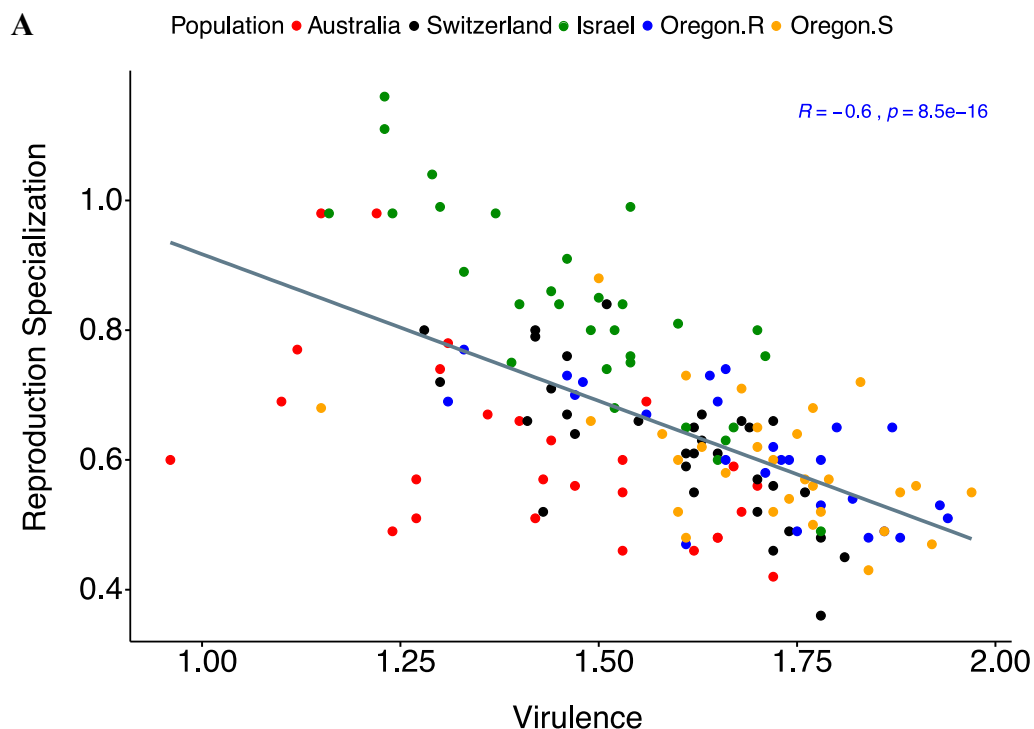
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318 *Host specialization reduces the mean trait performance*

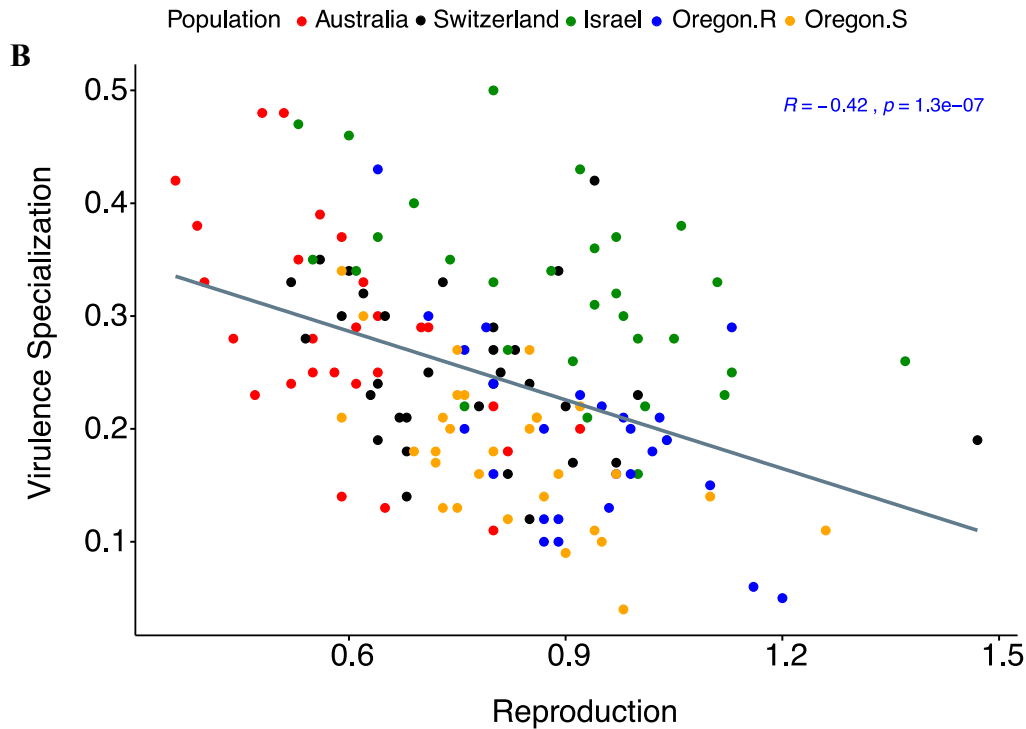
319 We investigated the influence of host specialization on the overall virulence and reproduction
320 and the maintenance of genetic diversity in the field. Using CV as a specificity metric, we
321 detected a significant negative correlation ($r = -0.60$, $P = 8.5e-16$, Fig. 3A) between
322 specialization for reproduction and mean virulence. This pattern indicates that isolates that are
323 generalists for reproduction have higher mean virulence across the 12 hosts. We also found a
324 significant negative correlation ($r = -0.42$, $P = 1.3e-07$, Fig. 3B) between specialization for
325 virulence and mean reproduction, indicating that isolates that exhibit host specialization for
326 virulence have an overall lower rate of reproduction across the 12 hosts. It is evident that most
327 of the isolates from the populations in Oregon and Israel are generalists or specialists,
328 respectively, for both traits (Figs. 3A & 3B). Importantly, the positive correlation ($r = 0.46$, P
329 $= 6.8e-09$, Fig. 4) between specialization for reproduction and maximum reproduction indicate
330 that there is a benefit associated with being a specialist.

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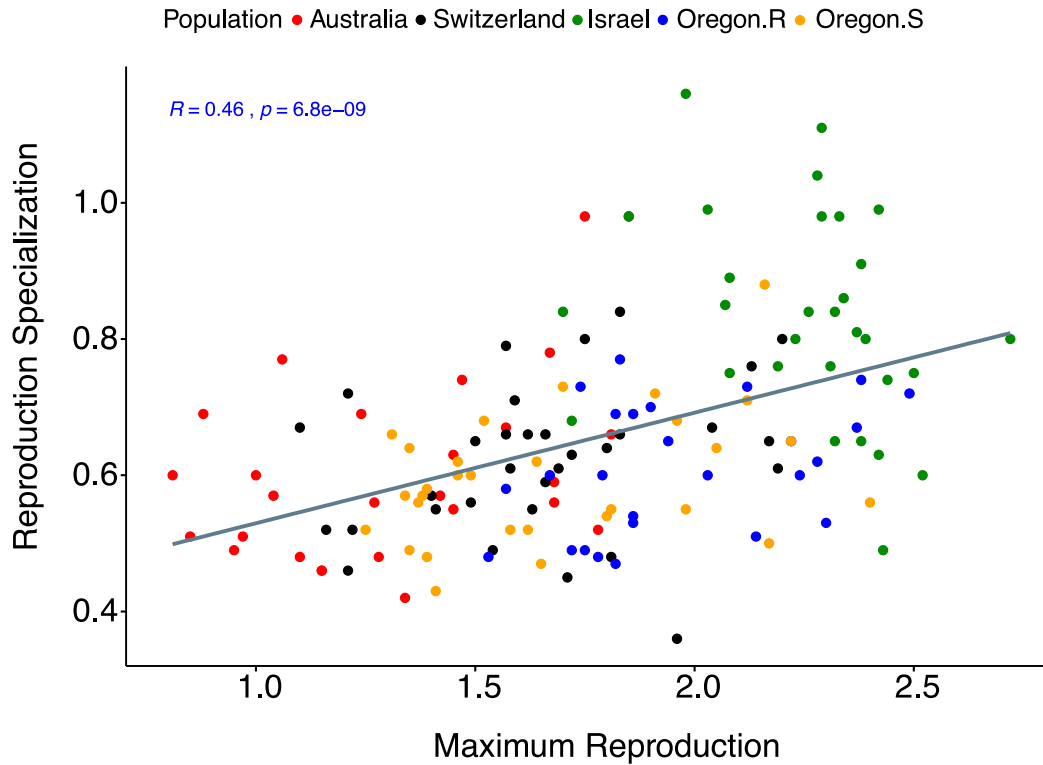
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334
335 **Figure 3.** Correlation between (A) overall mean virulence (amount of necrotic lesion area)
336 and reproduction specialization, (B) overall reproduction (pycnidia density within
337 lesions) and virulence specialization among 145 *Zymoseptoria tritici* isolates from five
338 populations. Specialization represents the estimates of coefficient of variation of
339 means across 12 hosts for each trait. Higher specialization indicates preference for
340 specific hosts to maximize trait performance. Data were log-transformed.



341

342 **Figure 4.** Correlation between maximum reproduction (maximum pycnidia density within
343 the lesion area produced by each isolate across 12 hosts) and reproduction
344 specialization (measured as the coefficient of variation of means for pycnidia density
345 within lesions across 12 hosts) among 145 *Zymoseptoria tritici* isolates from five
346 populations. Data were log-transformed.

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358 **Discussion**

359 Understanding the processes that maintain the variation in pathogenicity is of central
360 importance to predict pathogen evolution and understand disease dynamics (Burdon and Thrall,
361 2008). Using a panel of 12 diverse hosts and 145 pathogen strains, we demonstrated that
362 individual estimates of virulence and reproduction varied quantitatively, and that host genotype
363 and interactions with pathogen genotype are major contributors to the observed variation in
364 virulence and reproduction. We observed a strong positive correlation between the two traits
365 with substantial variation within each population. Furthermore, the data show a continuum of
366 host specialization among *Z. tritici* isolates, where isolates ranged between high and low
367 fecundity depending on the specific host infected. This may constrain directional evolution of
368 both virulence and fecundity and contribute to the maintenance of trait variation.

369

370 *Host diversity as a key determinant of pathogenicity trait variation*

371 Infection outcomes in many pathosystems are determined by genetic interactions among hosts
372 and pathogens (e.g. Pagan *et al.*, 2007; Pariaud *et al.*, 2009; Lannou, 2012; Tack *et al.*, 2012).
373 Earlier models of pathogen evolution often assumed that variation in infectivity is solely
374 controlled by pathogen strains (Restif and Koella, 2003). Here, in addition to the significant
375 host-isolate interactions, the effect of host cultivar on reproduction was almost 3-fold higher
376 than that observed for virulence, indicating that host genetic background strongly influences
377 resource allocation for reproduction following pathogen colonization (Vale *et al.*, 2011;
378 Attisano *et al.*, 2012). This result is consistent with previous studies, which show that even
379 after successful infection, host immune response, nutrient availability, and quantitative
380 resistance may hamper pathogen reproduction (Karisto *et al.*, 2018). The high level of
381 dependency on host genotype for reproductive fitness is consistent with results demonstrating
382 that different isolates maximize reproductive fitness on specific hosts and that host specific

383 patterns of fecundity are potentially important for the maintenance of pathogen diversity (see
384 discussion below).

385

386 *Evolution of pathogen virulence and fecundity in agroecosystems*

387 Here we show that highly virulent isolates of *Z. tritici* on average exhibit greater reproductive
388 potential. This likely reflects general links between pathogen induced necrosis and nutrient
389 release in this system (Zhan *et al.*, 2005; Kelm *et al.*, 2012). We did not detect any evidence
390 for a saturation point on the correlation curve generated from our dataset, suggesting that both
391 traits follow a continuous range. On a susceptible host, high levels of fecundity and virulence
392 are likely to be advantageous because highly virulent isolates can outcompete co-infecting
393 strains (Zhan *et al.*, 2015; McDonald and Stukenbrock, 2016). However, despite this seeming
394 advantage, highly virulent and fecund isolates did not dominate any population. Rather, we
395 observed that considerable variation for both virulence and fecundity is maintained within field
396 populations.

397

398 Trade-offs between transmission and virulence have been predicted to contribute to the
399 maintenance of variation for virulence. This reflects the assumption that increased virulence
400 decreases the longevity of the infected host so that the transmission period decreases, creating
401 a trade-off (Ebert and Bull, 2003; de Roode *et al.*, 2008). In this agricultural ecosystem
402 (characterized by high density monocropping with relatively homogeneous hosts), higher
403 reproduction (and higher virulence) may be predicted to increase transmission potential as the
404 reproductive units (pycnidia) contain spores that are dispersed by rain-splash throughout the
405 field, and hosts are replaced by farmers each growing season. However, it should be noted that
406 although fecundity can influence transmission, they are not synonymous. Under natural field
407 conditions, transmission depends on other factors such as the quantity, viability, and infection

408 efficiency of spores, as well as climatic conditions and, most importantly, the availability of a
409 susceptible host. How these traits and outcomes interact to determine virulence evolution is not
410 well understood.

411

412 We found considerable variation among populations in overall levels of virulence and
413 reproduction on a diverse set of hosts. We also found a strong correlation between these traits
414 within populations. These results suggest that selection for these traits can differ among
415 populations. One possible explanation for these findings is that the differential patterns of
416 virulence and reproduction reflect specific patterns of adaptation to the most common host
417 genotypes planted in the different wheat fields from which these pathogens were sampled.
418 However, the Swiss population displayed relatively low levels of virulence and fecundity,
419 which was surprising considering that the bulk of the tested hosts originated from Swiss
420 breeding programs. It is possible that the intensive breeding for STB resistance and frequent
421 introductions of new varieties from neighboring countries (Fossati and Brabant, 2003; Brabant
422 *et al.*, 2006) in Switzerland may have hampered selection for higher trait performance and
423 adaptation to a specific host. Thus, imposing diversifying selection on local pathogen
424 populations by recurrent changing of wheat cultivars and specific resistance traits might be a
425 useful strategy to disrupt evolution towards increased virulence and fecundity (Burdon *et al.*,
426 2014). Furthermore, in addition to quantitative resistance to *Z. tritici* (Yates *et al.*, 2019), wheat
427 varieties across the world often share common major resistance genes (Brown *et al.*, 2015),
428 which can be overcome by convergent evolution generating similar virulent mutations
429 independently in different geographical populations, even in the absence of gene flow among
430 populations (Croll and McDonald, 2017). These processes could explain the observed higher
431 performance of the Israeli and Oregon populations on Swiss hosts.

432

433 *Role of host specialization in the evolution of pathogenicity trait variation*

434 Does host specialization limit the emergence of “super-pathogen” strains combining higher
435 virulence, higher fecundity, and a broader host range? Here, all the hosts exhibited a wide
436 spectrum of resistance and susceptibility towards pathogen virulence and reproduction. For
437 example, the wheat genotypes 1011, Gene and Toronit showed greater resistance to virulence
438 and suppressed reproduction of some isolates, while other wheat genotypes were moderately
439 to highly susceptible to virulence and enabled higher reproduction of some isolates. Therefore,
440 we assessed patterns of host specificity by examining patterns of quantitative variation for
441 virulence and reproduction among the many host-isolate interactions in our experiment. We
442 found significant negative correlations between overall virulence and reproduction specificity,
443 indicating that isolates with high host specificity for reproduction (i.e. higher pycnidia
444 production on some hosts and a smaller number of pycnidia on other hosts) were on average
445 less virulent across all hosts (i.e. produced a lower average lesion area). This observation is
446 consistent with the hypothesis that increased specificity for fecundity results in decreased
447 virulence (Kirchner and Roy, 2002). This pattern further indicates a cost associated with host
448 specialization, which is suggested to result from antagonistic pleiotropy or non-overlapping
449 loci controlling trait performance on different hosts (Kawecki, 1994; Legros and Koella, 2010;
450 Hartmann *et al.*, 2017).

451

452 The observed positive relationship between specialization for reproduction and maximum
453 reproduction suggests that isolates following a generalist strategy fail to maximize their
454 reproductive fitness on a specific host, thus invoking the principle of “jack of all trades-master
455 of none” (Remold, 2012). This trade-off could explain why specialist isolates are maintained
456 within populations and why ‘super-pathogen’ strains do not dominate (Kassen, 2002). This is
457 consistent with the results reported by Thrall and Burdon (2003), where generalist isolates with

458 a broader host range had lower overall fecundity. Such specificity for reproduction may be
459 advantageous under scenarios involving host heterogeneity, competition during multiple
460 infection, and disruptive selection (Jaenike, 1990; Barrett *et al.*, 2009).

461

462 The precise scenario(s) generating heterogeneity, disruptive selection and promoting the
463 maintenance of variation for host specialization within and among populations remain
464 unknown. Specialists could outperform generalists on a given host (Garamszegi, 2006; Romero
465 and Elena, 2008) because generalists suffer from unequal selection pressure imposed by
466 different hosts (González *et al.*, 2019). For example, in a wheat field planted to a susceptible
467 cultivar (typically grown in a monoculture), being highly specialized in reproduction could be
468 beneficial because competition among isolates can favour selection for higher reproduction
469 instead of broader host range. In contrast, strains with high average levels of fecundity across
470 different host genotypes may be expected to have an advantage in environments with higher
471 levels of host genetic heterogeneity (May and Anderson, 1983; Frank, 1996). For example, in
472 areas where multiple cultivars are planted in relatively close proximity, generalist pathotypes
473 may have an advantage. Indeed, the maintenance of variation may reflect conflicts between
474 selection for fecundity and transmission within fields planted to a single susceptible cultivar
475 and selection for ability to infect multiple cultivars planted across an agricultural landscape. In
476 our case, whether the diversity of wheat host genotypes present within agricultural landscapes
477 is alone sufficient to explain the maintenance of generalist pathotypes given the presumed
478 advantage to specialists remains an interesting but open question.

479

480 **Conclusion**

481 Here, we report how different processes regulate life-history trait variation, which ultimately
482 improves our understanding of pathogen evolution and disease dynamics. Host diversity and

483 differential quantitative interactions with pathogen strains are likely key determinants of
484 variation in virulence and reproduction. Trade-offs for reproduction encountered by specialist
485 vs generalist isolates reinforce the general importance of costs in maintaining pathogenicity
486 trait variation. These mechanisms prevent the fixation of super-pathogen strains in pathogen
487 populations while indicating that diversifying the host in agricultural fields might be a useful
488 strategy to decelerate virulence evolution. Many approaches can be used to introduce dynamic
489 diversity into agricultural ecosystems and reduce selection for increased virulence (McDonald,
490 2014). A low tech approach that was shown in independent, replicated, field experiments to
491 impose diversifying selection on populations of three cereal pathogens is growing cultivar
492 mixtures (Zhan and McDonald, 2013). Implementing such dynamic diversity at the field scale
493 may impose such trade-offs on generalist isolates that lowers the ability to maximize
494 reproduction on any particular host (McDonald, 2014). We conclude that context-dependent
495 selective forces operating on pathogen populations located in different geographic locations
496 will likely play an ongoing role in contributing to the maintenance of variation in virulence and
497 reproduction in this and other pathosystems. While increased reproduction is likely to provide
498 more opportunities for transmission and virulence evolution in the environments that typify
499 much of modern agriculture, we still lack a deeper understanding of the impact of other life-
500 history traits that have an impact on epidemiology and disease dynamics, such as latent period,
501 spore quantity and infection efficiency.

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503 **Data availability**

504 Raw data used for this study are available at: <https://doi.org/10.5061/dryad.j3tx95x9m>.

505

506 **Author contributions**

507 LGB conceived the idea of the research. AD conducted the experiment, collected, and analyzed
508 the phenotypic data, wrote the manuscript with LGB. BAM and DC provided funding and
509 corrected the manuscript. All authors approved the final version of the manuscript.

510

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731 **Figures legends**

732 **Figure 1.** Multiple comparisons for (A) virulence (amount of necrotic lesion area) and (B)
733 reproduction (pycnidia density within lesions) among the five *Zymoseptoria tritici*
734 populations on 12 wheat hosts. Data were log-transformed.

735

736 **Figure 2.** Correlation between virulence (amount of necrotic lesion area) and reproduction
737 (pycnidia density within lesions; A) overall and (B) within each population, among 145
738 *Zymoseptoria tritici* isolates from five populations. Each point represents the overall
739 mean of virulence and reproduction combined over 12 hosts. Data were log-
740 transformed.

741

742 **Figure 3.** Correlation between (A) overall mean virulence (amount of necrotic lesion area)
743 and reproduction specialization, (B) overall reproduction (pycnidia density within
744 lesions) and virulence specialization among 145 *Zymoseptoria tritici* isolates from five
745 populations. Specialization represents the estimates of coefficient of variation of
746 means across 12 hosts for each trait. Higher specialization indicates preference for
747 specific hosts to maximize trait performance. Data were log-transformed.

748

749 **Figure 4.** Correlation between maximum reproduction (maximum pycnidia density within
750 the lesion area produced by each isolate across 12 hosts) and reproduction
751 specialization (measured as the coefficient of variation of means for pycnidia density
752 within lesions across 12 hosts) among 145 *Zymoseptoria tritici* isolates from five
753 populations. Data were log-transformed.

