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Title: ~~Interactions of T~~tomato and *Botrytis* genetic diversity: Parsing the contributions of host differentiation, domestication and pathogen variation~~Crop domestication TDomesticated tomato dDomestication and pathogen virulence: Interactions of tomato host and *Botrytis* genetic diversity~~

Short title: Interactions of tomato and Botrytis genetics

Abstract

~~Human selection during crop domestication alters numerous traits, including disease resistance.~~ While the impact~~s~~ of ~~these processes~~ crop domestication on specialist pathogens are well-studied, less~~Little~~ is known about ~~how~~ the interaction of crop variation and generalist pathogens~~crop domestication variation affects quantitative interactions with generalist pathogens~~. To study how ~~genetic variation within a domesticate~~standing genetic variation ~~and domestication~~ within a~~d~~ crop ~~domestication~~ impacts plant resistance to generalist pathogens, we infected a collection of wild and domesticated tomato accessions with a genetically diverse population of the generalist pathogen *Botrytis cinerea*. ~~and to uncover variants in the correspondingly how this interacts with the pathogen's genetics, we infected a collection of wild and domesticated tomato accessions with a genetically diverse population of the generalist pathogen Botrytis cinerea.~~ We quantified variation in lesion size of 97 *B. cinerea* genotypes (isolates) on 6 domesticated *Solanum lycopersicum* and 6 wild *S. pimpinellifolium* genotypes. Lesion size was significantly ~~generated~~affected by large effects of the host and pathogen's genotype, with a much smaller contribution of domestication ~~controlled by plant domestication, plant genetic variation, and the pathogen's genotype~~. This pathogen collection also enables ~~Overall, resistance was slightly elevated in the wild tomato accessions. G~~genome-wide association (GWA) mapping in *B. cinerea*. GWA in the pathogen showed that virulence is ~~identified a~~ highly polygenic ~~collection~~ and involves a diversity of mechanisms~~of genes~~. ~~B~~This suggests that ~~b~~reeding against this pathogen would likely need to utilize ~~a diversity of~~diverse isolates to capture all possible mechanisms. Critically, we identified a ~~discrete~~ subset of *B. cinerea* genes where ~~the~~ allelic variation was linked to altered virulence against the wild versus domesticated tomato ~~accessions~~, as well as loci that could handle both groups. T~~his indicates that t~~his generalist pathogen already has ~~the necessary~~a large collection of allelic variation ~~in place to handle the introgression of wild resistance mechanisms into the domesticated crop~~that must be considered when designing a breeding program.

Introduction

Plant disease is mediated by complex interactions among diverse host and pathogen molecular pathways, and the disease outcome is the sum of host plant susceptibility/resistance and pathogen virulence/sensitivity mechanisms. The specific outcome of any interaction is highly dependent on the genetic variation within these pathways in both the host and pathogen. Over time, mutation and selection have led to distinct genetic architectures in the host and pathogen that are at least partly influenced by the host range of the pathogen. Specialist pathogens are a major focus in plant pathology; virulent on a narrow range of hosts, and often limited to a single species or genus. Most known plant genes for resistance to specialist pathogens confer qualitative resistance through innate immunity via large-effect loci that enable the recognition of the pathogen (Dangl and Jones 2001, Jones and Dangl 2006, Dodds and Rathjen 2010, Pieterse, Van der Does et al. 2012). These recognition signals can be conserved pathogen patterns such as cell-wall polymers or flagellin, or alternatively, specific virulence factors that block perception of the pathogen, and in turn are detected by plant proteins that guard the signaling networks (Jones and Dangl 2006, Bittel and Robatzek 2007, Ferrari, Galletti et al. 2007, Boller and He 2009, Dodds and Rathjen 2010). The evolution of large-effect qualitative loci has partly been driven by the narrow host range for the pathogen that enhances co-evolution between host resistance genes and pathogen virulence mechanisms.

In contrast to specialist pathogens, generalist pathogens are virulent across a wide range of plant host species. Generalist pathogens potentially have less stringent co-evolution to specific hosts and their accompanying resistance mechanisms, because these pathogens can easily shift to new hosts in the environment. This allows generalist pathogens to evade the rapid evolution of new resistance mechanisms within specific hosts until they evolve to counter this new resistance. This niche-shifting ability may partially explain the observation that most natural resistance to generalist pathogens is highly polygenic, and the underlying plant genes for resistance are quantitative (Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Rowe and Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016). Plant quantitative resistance genes to generalist pathogens include a broad array of direct

85 defense genes, like those involved in secondary metabolite production, cell wall

86 formation, and defense proteins (Zhang, Khan et al. 2002, Denby, Kumar et al. 2004,

87 Zipfel, Robatzek et al. 2004, Ferrari, Galletti et al. 2007, Rowe and Kliebenstein 2008,

88 Poland, Balint-Kurti et al. 2009, Corwin, Copeland et al. 2016). Importantly, these

89 quantitative plant resistance loci do not alter resistance to all genotypes (isolates) of a

90 pathogen but interact with the infecting pathogen's genotype. For example, the ability of

91 the *Arabidopsis* defense metabolite, camalexin, to provide resistance to *Botrytis cinerea*

92 depends upon whether the specific isolate is sensitive or resistant to camalexin

93 (Kliebenstein, Rowe et al. 2005, Pedras and Ahiahonu 2005, Stefanato, Abou-Mansour

94 et al. 2009, Pedras, Hossain et al. 2011) and similarly *B. cinerea* virulence on tomato

95 varies with the isolate's ability to detoxify tomatine (Quidde, Osbourn et al. 1998,

96 Quidde, Büttner et al. 1999). In contrast to the polygenic nature of plant resistance to

97 generalist pathogens, little is known about the genetic architecture of virulence within

98 generalist pathogens, and how this is affected by genetic variation in the plant (Bartoli

99 and Roux 2017). There are no reported naturally variable large-effect virulence loci in

100 generalist pathogens, suggesting that virulence in generalist pathogens is largely

101 quantitative and polygenic. This potential for interaction between polygenic virulence in

102 generalist pathogens and equally polygenic resistance in host plants suggests that we

103 need to work with genetic variation in both the host and pathogen to truly understand

104 quantitative host-pathogen interactions.

105 A Domestication of crop plants is a key evolutionary process in plants that has

106 affected resistance to specialist pathogens is the domestication of crop plants.

107 Domesticated plant varieties are typically more sensitive to specialist pathogens than

108 their wild relatives (Smale 1996, Rosenthal and Dirzo 1997, Couch, Fudal et al. 2005,

109 Dwivedi, Upadhyaya et al. 2008), and pathogens may evolve higher virulence on

110 domesticated hosts (Stukenbrock and McDonald 2008). Further, domestication typically

111 imposes a genetic bottleneck that reduces genetic diversity in the crop germplasm,

112 including decreased availability of resistance alleles against specialist pathogens

113 (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Chaudhary 2013). These

114 general evolutionary patterns, of lower reduced resistance and allelic diversity found

115 when studying the interaction of specialist pathogens with crop plants, are assumed to

116 ~~similarly~~ hold for generalist pathogens and their domesticated hosts. However, there is

117 less information about how crop host domestication affects disease caused by

118 generalist pathogens, when the resistance to these pathogens is quantitative and

119 polygenic rather than qualitative and monogenic. As such, there is a need to quantify

120 the effect of domestication on a broad generalist pathogen in comparison to the rest of

121 the crop's standing variation to test ~~conduct a detailed analysis of~~analyze how

122 ~~domestication may alter the interaction of a plant with a broad generalist pathogen, and~~

123 ~~correspondingly,~~ how and if ~~how~~ domestication influences the pathogen.

124   *Botrytis cinerea* provides a model generalist pathogen for studying quantitative

125 interactions with plant hosts and underlying evolutionary processes. ~~for this generalist~~

126 ~~in contrast to specialist pathogens.~~ *B. cinerea* is a broad generalist pathogen that can

127 infect most tested plants, from bryophytes to eudicots, and causes wide ranging pre-

128 and post-harvest crop losses (Nicot and Baille 1996, Elad, Williamson et al. 2007,

129 Fillinger and Elad 2015). Individual isolates of *B. cinerea* show the same broad host

130 range ~~(Deighton, Muckenschnabel et al. 2001, Finkers, van Heusden et al. 2007, Ten~~

131 ~~Have, van Berloo et al. 2007, Corwin, Subedy et al. 2016),~~ (Deighton, Muckenschnabel

132 et al. 2001, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007,

133 Corwin, Subedy et al. 2016). This is in contrast to pathogens like *Fusarium oxysporum*

134 where the species can infect diverse hosts, but each isolate is highly host specific

135 (Katan 1999, Ormond, Thomas et al. 2010, Loxdale, Lushai et al. 2011, Barrett and Heil

136 2012). *B. cinerea* isolates display significant variation in virulence phenotypes, partly

137 due to genetic variation in specific virulence mechanisms, like the production of the

138 phytotoxins, botrydial and botcinic acid (Siewers, Viaud et al. 2005, Dalmais,

139 Schumacher et al. 2011). This genetic variation also influences cell wall degrading

140 enzymes and key regulators of virulence like *VELVET* that quantitatively control

141 virulence on multiple host plants (Rowe and Kliebenstein 2007, Schumacher, Pradier et

142 al. 2012). ~~This genetic variation in diverse~~This standing diversity in virulence

143 mechanisms can contribute to the formation of quantitative differences in virulence

144 between the isolates (ten Have, Mulder et al. 1998). The phenotypic variation is driven

145 by a high level of sequence diversity spread across the genome (Rowe and

146 Kliebenstein 2007, Fekete, Fekete et al. 2012). The polymorphism rate in *B. cinerea*

147 ~~was measured as~~, 376~~.6~~ SNP/kb, ~~which~~ is much more variable than most previously

148 studied plant pathogens (1-2 SNP/kb in *Blumeria graminis*, 1.5 SNP/kb in *Melampsora*

149 *larici-populina*, 5.5 SNP/kb in the compact genome of the obligate biotroph

150 *Plasmodiophora brassicae*, 12.3 SNP/kb in the wheat stem rust pathogen *Puccinia*

151 *graminis* f. sp. *tritici*) and human pathogens (3-6 SNP/kb in *Mycobacterium*

152 *tuberculosis*), ~~and close to the genetic diversity~~that ~~found in the human pathogen~~

153 ~~*Mycobacterium tuberculosis* (2.9 to 6.2 SNP/kb)~~ (Farhat, Shapiro et al. 2013, Hacquard,

154 Kracher et al. 2013, Wicker, Oberhaensli et al. 2013, Persoons, Morin et al. 2014,

155 Upadhyaya, Garnica et al. 2014, Atwell, Corwin et al. 2015, Desjardins, Cohen et al.

156 2016, Power, Parkhill et al. 2017, Atwell, Corwin et al. 2018)~~. Higher polymorphism~~

157 ~~rates are reported for the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici*, from~~

158 ~~a small non-random sample of isolates (12.3 SNP/kb) (Upadhyaya, Garnica et al. 2014)~~.

159 In addition to SNP diversity, the genomic sequencing showed that *B. cinerea* has a high

160 level of recombination and genomic admixture, as if it were a randomly intermating

161 population (Supplemental Figure 1) (Atwell, Corwin et al. 2018). As such, a collection of

162 *B. cinerea* isolates contain~~s~~ genetic variation in a wide range of virulence mechanisms,

163 offering the potential to challenge the host with a blend of diverse virulence

164 mechanisms~~.~~ to identify the pathogen variation controlling quantitative virulence

165 (Corwin, Subedy et al. 2016, Bartoli and Roux 2017, Atwell, Corwin et al. 2018,

166 Fordyce, Soltis et al. 2018)~~. This can potentially identify the pathogen variation~~

167 ~~controlling quantitative virulence, even in non-model plant systems (Bartoli and Roux~~

168 ~~2017)~~.

169 A model pathosystem for studying quantitative host-pathogen interactions ~~during~~

170 ~~domestication~~ is the tomato-*B. cinerea* system, where the pathogen causes crop loss

171 due to both pre- and post-harvest infection (Dean, Van Kan et al. 2012, Hahn 2014,

172 Romanazzi and Droby 2016). Resistance to *B. cinerea* is a quantitative trait in tomato

173 as with most other species, with identified tomato QTLs each explaining up to 15% of

174 phenotypic variation for lesion size on stems (Dıaz, ten Have et al. 2002, Finkers, van

175 Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Rowe and Kliebenstein 2008,

176 Corwin, Copeland et al. 2016). Tomato is also a key model system to study how

177 domestication influences plant physiology and resistance, including alterations in the

178　circadian clock (Tanksley 2004, Bai and Lindhout 2007, Panthee and Chen 2010,

179　Bergougnoux 2014, Müller, Wijnen et al. 2016), which can modulate resistance to *B.*

180　*cinerea* (Sauerbrunn and Schlaich 2004, Weyman, Pan et al. 2006, Bhardwaj, Meier et

181　al. 2011, Hevia, Canessa et al. 2015). This suggests that host plant ~~domestication~~

182　diversity within tomato can alter traits known from other systems to influence *B. cinerea*

183　resistance ~~from other systems~~. Tomato domestication is typically considered a single

184　event, followed by extensive crop improvement (Lin, Zhu et al. 2014, Blanca, Montero-

185　Pau et al. 2015). Thus, we are using the tomato-*B. cinerea* pathosystem to directly

186　measure the interaction of domesticated crop ~~domestication~~ variation with genetic

187　variation in a generalist pathogen to better understand the evolution of this

188　pathosystem.

189　　　　In this study, we infected 97 genetically diverse *B. cinerea* isolates on a collection

190　of domesticated tomato, *S. lycopersicum*, and wild tomato, *S. pimpinellifolium*, and

191　quantified the interaction through lesion size in a detached leaf assay. Previous studies

192　have examined *B. cinerea* resistance between domesticated and ~~distantly related~~ wild

193　tomato species ~~(i.e. S. lycopersicum and S. pimpinellifolium)~~ using single isolates of

194　pathogens (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002, Guimaraes,

195　Chetelat et al. 2004, Ten Have, van Berloo et al. 2007, Finkers, Bai et al. 2008). These

196　previous studies typically used individual wild and domesticated tomato accessions that

197　were the founders of mapping populations and found a wide range of *B. cinerea*

198　resistance. However, it is still unknown how domesticated and ~~closely related~~ wild

199　tomatoes compare for *B. cinerea* resistance using multiple plant genotypes and a

200　population of the pathogen. We selected accessions to sample major geographic origins

201　of the progenitor species, and focused the domesticated germplasm on diverse mid- to

202　late- 20th century improved germplasm (Lin, Zhu et al. 2014, Blanca, Montero-Pau et al.

203　2015). In this study, we asked whether *B. cinerea* virulence was controlled by host

204　variation, pathogen variation, or the interaction between them. Lesion size of *B. cinerea*

205　is a quantitative trait that was controlled by plant domestication status, plant genotype

206　and pathogen isolate. ~~We looked for evidence of specialization within our generalist~~

207　~~pathogen population. While our B. cinerea isolates appear to be generalists across~~

208　~~domestication in Solanum, a subset of isolates is sensitive to tomato domestication.~~

209   Finally, we aimed to identify the genetic basis of variation in *B. cinerea* virulence on

210   ~~domesticated and wild tomato~~*S. lycopersicum* and *S. pimpinellifolium*. We conducted

211   genome-wide association (GWA) in *B. cinerea* to identify pathogen loci where genetic

212   variation leads to altered virulence across the host genotypes, including a specific test

213   for loci that influence responses to crop domestication. Few studies have conducted

214   GWA in plant pathogens for virulence phenotypes, and most of these were limited by

215   few variable loci or few genetically distinct isolates (Dalman, Himmelstrand et al. 2013,

216   Gao, Liu et al. 2016, Talas, Kalih et al. 2016, Wu, Sakthikumar et al. 2017). <u>Our</u>

217   <u>previously-sampled isolate collection includes genetic diversity across 272,672 SNPs</u>

218   <u>(Supplemental Figure 1)</u>~~(Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017)~~ (Atwell,

219   Corwin et al. 2015, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018)<u>.</u> ~~To ensure~~

220   ~~that genetic inference was independent of the GWA method or SNP diversity reference,~~

221   ~~we repeated genetic analysis with two different association methods (bigRR and~~

222   ~~GEMMA) using SNPs called in comparison to two published *B. cinerea* genomes (T4~~

223   ~~and B05.10). All methods converged on the same image of genetic architecture;~~ <u>We</u>

224   <u>found that the genetic architecture of</u> virulence of *B. cinerea* is highly quantitative, with

225   hundreds of significant SNPs with small effect sizes associated with lesion area on each

226   tomato genotype. Importantly, there is a subset of loci in the pathogen where allelic

227   variation gives the isolates opposing responses to crop domestication. These pathogen

228   loci could provide tools for understanding how domestication in tomato has influenced

229   generalist pathogen resistance, to inform breeding efforts.

230

231   **Results**

232   **Experimental Design**

233   To measure how tomato ~~domestication~~ <u>genetic variation</u> affects quantitative

234   resistance to a population of a generalist pathogen, we infected a collection of 97

235   diverse *B. cinerea* isolates (genotypes) on wild and domesticated tomato

236   genotypes~~(Zhang, Corwin et al. 2017)~~. ~~We compared domesticated and closely related~~

237   ~~wild tomatoes for *B. cinerea* resistance using multiple plant genotypes and a population~~

238   ~~of the pathogen.~~ We selected 6 domesticated *Solanum lycopersicum* and 6 wild *S.*

239   *pimpinellifolium* accessions, the closest wild relative of *S. lycopersicum*, to directly study

240 how domestication has influenced resistance to *B. cinerea* (Peralta, Spooner et al.

241 2008, Müller, Wijnen et al. 2016)(Supplemental Figure 21). Our previously collected *B.*

242 *cinerea* sample includes 97 isolates obtained from various eudicot plant hosts, including

243 tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2,

244 Supersteak)~~(Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017)~~ (Atwell, Corwin et al.

245 2015, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018). We infected all 97 *B.*

246 *cinerea* isolates onto each of the 12 plant genotypes in 3-fold replication across 2

247 independent experiments in a randomized complete block design, giving 6

248 measurements per plant-pathogen combination, for a total of 3,276 lesions. Digital

249 measurement of the area of the developing lesion provides a composite phenotype

250 controlled by the interaction of host and pathogen genetics. This measurement of the

251 plant-*B. cinerea* interaction has been used successfully in a number of molecular and

252 quantitative genetic studies (Ferrari, Plotnikova et al. 2003, Denby, Kumar et al. 2004,

253 Kliebenstein, Rowe et al. 2005, Ferrari, Galletti et al. 2007, Ten Have, van Berloo et al.

254 2007, AbuQamar, Chai et al. 2008, Rowe and Kliebenstein 2008, Liu, Hong et al. 2014).

255 It should be noted that we are not focusing on MAMP or PAMP specific host/pathogen

256 interactions with this study, we are instead allowing the identification of any mechanism

257 that may influence the host/pathogen interaction including metabolism, development or

258 any other unknown component. If there is genetic variation affecting the trait, and the

259 trait influences the interaction of host and pathogen, it will be a component of the

260 experiment. This fits with the recently developing view that growth, development and

261 resistance in plants are highly integrated processes that may not be as distinct as once

262 believed (Campos, Yoshida et al. 2016, Ballaré and Pierik 2017, Züst and Agrawal

263 2017, Izquierdo-Bueno, González-Rodríguez et al. 2018).

264

265 **Lesion size (phenotypic) variation**

266 We collected images of all lesions at 24, 48, and 72 hours post inoculation. At 24 hours,

267 no visible lesions were present on the tomato leaves. At 48 hours, a thin ring of primary

268 lesion became visible surrounding the location of the spore droplet, but no expansion

269 was visible. At 72 hours significant lesion growth was visible, but no lesions had spread

270 to infect over half of the leaflet. We digitally measured the area of all developing lesions

at 72 hours post infection (HPI) as a measure of virulence (Figure 1). We use the linear measurement of lesion area for several reasons. First, ~~Accordin~~in previous work ~~to previous studies, at~~ 72 HPI *B. cinerea* lesion area growth appears to enter a relatively linear growth phase (Rowe, Walley et al. 2010)~~,~~. Secondly, ~~and~~ previous research ~~from our group~~ has shown that the linear measurement behaves as a normally distributed trait ~~modeled lesion area as a linear or log-linear trait~~~~(Kliebenstein, Rowe et al. 2005, Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018)~~ (Kliebenstein, Rowe et al. 2005, Corwin, Copeland et al. 2016, Atwell, Corwin et al. 2018, Fordyce, Soltis et al. 2018). And finally, previous work has shown that Botrytis isolates display large variation in their unit biomass per ~~area~~ lesion area and as such growth in biomass is not the sole factor driving this measure~~Lesion area is a common measure of the interaction of plant immunity and pathogen virulence, though pathogen biomass does not scale with lesion area~~ (Corwin, Subedy et al. 2016)~~(Rowe, Walley et al. 2010, Corwin, Copeland et al. 2016)~~ We observed a mean lesion size of 0.67 cm^2 across the full experiment, with 0.94 CV across the full isolate population on all tomato genotypes. Individual isolates were highly variable in their lesion size across tomato genotypes (Figure 1 c-h), with mean lesion size per isolate of 0.14 cm^2 to 1.29 cm^2, and individual isolate coefficient of variation (CV) from 0.51 to 1.68 across all observations on all tomato genotypes (Supplemental Data Set 1). A subset of these isolates is highly virulent on tomato (mean lesion size > 1.05 cm^2, Figure 1e), and a subset can be considered saprophytic (mean lesion size < 0.3 cm^2, Figure 1f). Lesion size of *B. cinerea* on tomato ~~was weakly~~showed a weak positive correlat~~ed~~ion with lesion size on *A. thaliana* from previous studies; both on domesticated tomato (r=0.247, p= 0.003) and on wild tomato (r=0.301, p= 0.016) (Supplemental Figure 3)(Zhang, Corwin et al. 2017). This lack of correlation s~~S~~uggest~~sing~~ the presence of~~at there are~~ both shared and unique mechanisms of quantitative virulence i~~o~~n the two species.

Contribution of Pathogen Genetics~~,~~ and Plant Genetics ~~and Crop Domestication~~ Effects on Resistance

301 To measure the relative contribution of genetic diversity in the plant and the pathogen to

302 variation in the virulence/ susceptibility phenotype, we used a ~~multiple linear~~

303 ~~regression~~general linear model ~~(R Development Core Team 2008)~~ (R lme4

304 package;(Bates, Maechler et al. 2015)). This model directly tested the contribution of

305 pathogen genotype (isolate), plant genotype, and plant domestication status~~, and~~

306 ~~pathogen genotype (isolate)~~ to variation in lesion size. The final model showed that

307 genetic variation within both the host plant and the pathogen had significant effects on

308 lesion growth, ~~with pathogen isolate diversity explaining 3.5 fold4 more variance than~~

309 ~~plant genotype~~each explaining approximately the same portion of the variance ~~, 46% of~~

310 ~~total genetic variance for pathogen isolate vs. 13% for plant genotype~~ (Table 1 and

311 Figure 1c). Interestingly, while tomato domestication status significantly impacted *B.*

312 *cinerea* virulence, ~~as~~ it was to a much lower level than the other factors~~shown by the~~

313 ~~small but significant effects of genetic variation between domesticated and wild~~

314 ~~tomatoes~~ (~~3.5% of total genetic variance,~~Table 1). There was no evidence for

315 significant interaction effects between pathogen isolate and plant genotype~~, but this~~

316 ~~term contributed the largest proportion of the plant-related variance in lesion size (34%~~

317 ~~of total genetic variance, Table 1). The lack of significance for this term in face of the~~

318 ~~large fraction of variance may be due to the vast degrees of freedom in this term (Table~~

319 ~~1).~~. Thus, the interaction between tomato and *B. cinerea* was significantly controlled by

320 genetic diversity within the host plant and the pathogen, including a slight effect of

321 domestication status.

322

323 **Table 1. ANOVA results of the interaction between 12 tomato accessions and 95**
324 ***B. cinerea* isolates measured as lesion area.**
325 Results of general linear modelling of lesion area for 12 tomato accessions by 95 *B.*
326 *cinerea* isolates is shown (R lme4 package version 1.1-18-1;(Bates, Maechler et al.
327 2015)). Two of our 97 isolates did not have replication across 2 experiments, so they
328 were dropped at this stage of analysis. The terms are as follows; Isolate is the 95 *B.*
329 *cinerea* isolates, Domestication is wild tomato, *S. pimpinellifolium*, versus domesticated
330 tomato, *S. lycopersicum*, Plant is 12 tomato genotypes nested within their respective
331 domestication groupings, Experiment tests the random effect of 2 independent replicate
332 experiments. The nested random effects of whole plant sampled, leaf sampled, and
333 leaflet pair are included. In addition, interactions of these factors are tested (:). The
334 degrees of freedom and p-value are shown. For fixed effects, the type II sum of squares
335 and F-value are shown, and for random effects the likelihood ratio test statistic (LRT) is
336 shown.

337

Fixed Effect	SS	F value	DF	p
Isolate	37.8	1.7	94	**0.007**
Domestication	3.4	14.1	1	**0.0006**
Domest/Plant	39.3	16.2	10	**5e-11**
Iso:Domest	15.8	0.7	94	0.99
Iso:Domest/Plant	179.1	0.8	940	1
Random Effect	LRT	DF	p	
1 | Experiment	136	1	**<2e-16**	
1 | Whole Plant	0.21	1	0.65	
1 | WP/Leaf	22.4	1	**2e-06**	
1 | WP/Leaf/Leaflet Pair	0	1	1	
1 | Exp:Iso	321	1	**<2e-16**	

338

Pathogen Specialization to Source Host

One evolutionary model of plant-generalist pathogen interactions suggests that generalist pathogen isolates within ~~a~~ a generalist species may specialize for interaction with specific hosts. Alternatively, generalist isolates may show no host specialization or preference. Our collection of *B. cinerea* includes five isolates that may be adapted to tomato, as they were collected from *S. lycopersicum*. To test if there is evidence for specialization to the source host, we compared the virulence of the *B. cinerea* isolates obtained from tomato to the broader pathogen population. For *B. cinerea* genotypes isolated from tomato tissue vs. other hosts, there was no significant difference in lesion size ~~on domesticated tomato (t-test; t=1.10, n = 97, p=0.33), wild tomato (t-test; t=1.09, n = 97, p=0.33) or~~ across all tomato genotypes (t-test; n = 97, p=0.14) (Figure 1g). In fact, one isolate collected from tomato tissue (KGB1) was within the 10 least-virulent isolates and another (Triple3) was within the 10 most-virulent isolates (Figure 1g). This demonstrated significant genetic variation in virulence across the *B. cinerea* isolates, and that this collection of *B. cinerea* isolates from tomato do not display a strong host-specificity for tomato (Martinez, Blancard et al. 2003, Ma and Michailides 2005, Rowe and Kliebenstein 2007, Samuel, Veloukas et al. 2012).

Pathogen Specialization to Host Genotype

358 Though we did not find evidence for *B. cinerea* ~~adaptation to~~preference for tomato

359 based on isolate host source, the *B. cinerea* isolates may contain genetic variation at

360 individual loci that allow them to better attack subsets of the tomato genotypes (Rowe

361 and Kliebenstein 2007, Kretschmer and Hahn 2008, Corwin, Subedy et al. 2016). A

362 visual analysis of the data suggested an interaction between the genomes of *B. cinerea*

363 and tomato (Figure 1 c-h). However, when using the full model, we found no significant

364 interaction between isolate and individual host genotype, even though there was a large

365 fraction of variance within these terms (Table 1). This may indicate a lack of interaction

366 between genetic variation in the host and pathogen. Interaction effects in large datasets

367 can be difficult to identify using mixed models, so we used a second standard statistical

368 approach, a Wilcoxon signed-rank test~~,~~. ~~to~~ We used model-adjusted lesion sizes as

369 input to test if the rank of *B. cinerea* isolate-induced lesion size significantly changes

370 between pairs of tomato genotypes. This showed that when using the full isolate

371 population, the rank performance of the isolates does significantly vary between host

372 genotypes. When comparing mean lesion size between paired plant genotypes, ~~58~~59%

373 (~~38~~ 39 out of 66) of tomato accession pairs had significantly different ranking of the

374 isolates (Wilcoxon signed-rank test with Benjamini-Hochberg FDR-correction, Table 2,

375 Supplemental Figure ~~4~~2). A significant p-value indicates that the two host genotypes

376 show evidence for different virulence interactions with the population of *B. cinerea*

377 isolates, providing evidence for host x pathogen genotypic interactions. This pattern was

378 consistent across domesticated host pairs, wild host pairs, or between-species host

379 pairs (Wilcoxon signed-rank test with B-H FDR-correction, Table 2). This provides

380 evidence~~suggests~~ that the population of *B. cinerea* does display differential responses

381 to the tomato genetic variation.

382 To focus on whether specific *B. cinerea* isolates may be sensitive to tomato

383 domestication, we applied a Wilcoxon and ANOVA approach. Overall, most isolates

384 (78/97, 80%) are more virulent on domesticated than wild tomato (Figure 3;

385 Supplemental Data Set 1). Using a ~~The~~ Wilcoxon signed-rank test~~,~~ to compare the rank

386 of model-corrected mean lesion size of all the *B. cinerea* isolates on wild versus

387 domesticated tomato~~, was showed~~we found a significant difference~~significant~~ (Wilcoxon

388 signed-rank test, W = ~~5946~~5801, p-value = 0.~~002~~0007) (Figure 3). While this shows a

general population behavior, we used single-isolate ANOVAs ~~To~~ to ~~identify~~ test if any ~~specific~~the pathogen genotypes ~~most~~ had a significant ~~sensitive to~~strongly associated ~~with domestication~~association with domestication. ~~, we conducted single-isolate ANOVAs on~~These general linear models ~~including~~ included the fixed effects of plant, domestication, and the random effect of experiment~~,~~. After adjusting for multiple testing, this identified~~and found~~ two isolates (Fd2, Rose) with a significant effect of domestication on lesion size ($p < 0.05$, FDR corrected) (Figure 1h), both of which are more virulent on domesticated tomato (Supplemental Data Set 3). ~~These included one of the highly virulent isolates (Fd2), and one of the largely saprophytic isolates (Rose), which suggests that isolate virulence level on tomato does not predict *B. cinerea* genetic response to tomato domestication. Both of these isolates were more virulent on domesticated than on wild tomato. These results suggest that this *B. cinerea* population contains two highly domestication-sensitive associated isolates which are more virulent on domesticated tomato, and a broader pattern of *B. cinerea* sensitivity to tomato genetic variation.~~

To assess whether isolates could appear domestication-associated due to random chance, we bootstrapped assignment of plant accessions to domestication groups. ~~Across the 100 bootstraps, we~~96 of the 100 bootstraps identified no isolates with domestication sensitivity, and the other four bootstraps identified only ~~identified~~ 2 isolates showing significant domestication association (FDR <0.01)~~to domestication in 4% of the random datasets~~. Therefore, our individual isolate observations are in the 96th percentile. ~~This~~While this is suggestive, ~~suggests that~~ a more precise estimate of isolate x domestication interactions would require larger experiments using either more replication or additional plant genotypes.

Table 2. Rank order shifts of 97 *B. cinerea* isolates by lesion area across all of the tomato accessions.
Wilcoxon signed-rank test on comparing model-corrected mean *B. cinerea* lesion area on tomato accessions. This tests for a change in the rank order of the 97 isolates between each pair of tomato accessions. A significant p-value suggests that the relative performance of individual isolates is altered from one host to the other. The lower left corner of the chart includes B-H FDR-corrected p-values, the upper right corner includes the test statistic (W). Bold text indicates significance at $p < 0.01$ after correction, italicized text indicates suggestive p-values $0.01 < p < 0.1$. NS shows non-significant interactions.

424

		Wild						Domesticated					
		LA1547	LA1589	LA1684	LA2093	LA2176	LA480	LA2706	LA3008	LA3475	LA410	LA4345	LA4355
Wild	LA1547		2978	3988	2927	1865	3008	1710	3460	1597	1135	3928	2944
	LA1589	<0.001		5401	4699	3359	4662	3014	4918	2938	2340	5536	4454
	LA1684	NS	0.029		3709	2552	3690	2296	4004	2205	1690	4537	3571
	LA2093	<0.001	NS	0.049		3013	4496	2732	4889	2588	1947	5534	4264
	LA2176	<0.001	0.004	<0.001	<0.001		5837	4029	6002	3963	3276	6706	5583
	LA480	<0.001	NS	0.044	NS	0.001		6143	4192	6286	6855	3575	4702
Domesticated	LA2706	<0.001	<0.001	<0.001	<0.001	NS	<0.001		6311	4523	3876	6917	5940
	LA3008	0.009	NS	NS	NS	<0.001	NS	<0.001		2619	2082	5100	4049
	LA3475	<0.001	<0.001	<0.001	<0.001	NS	<0.001	NS	<0.001		3815	7088	5984
	LA410	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	NS	<0.001	NS		7567	6602
	LA4345	0.16	0.011	NS	0.011	<0.001	0.021	<0.001	NS	<0.001	<0.001		3439
	LA4355	<0.001	NS	0.02	NS	0.008	NS	<0.001	NS	<0.001	<0.001	<0.001	

425

426

Domestication and Lesion Size Variation

Existing literature predominantly reports that crop domestication decreases plant resistance to pathogens (Smale 1996, Rosenthal and Dirzo 1997, Couch, Fudal et al. 2005, Dwivedi, Upadhyaya et al. 2008, Stukenbrock and McDonald 2008). While we did observe the expected decreased resistance (by 18%) in domesticated tomato (Figure 2 and 3, Table 1), domestication was a minor player in controlling lesion size variation, with most of the plant genetic signature coming from variation within both the wild and domesticated tomato species, contributing 12-fold more variation in resistance than domestication alone (Table 1). . In our analysis, we identified a significantly greater (18%) resistance of wild tomato in comparison to domesticated tomato across the

15

population of *B. cinerea* isolates (Figure 2 and 3, Table 1). ~~However, this domestication effect was not the dominant source of variation, as genetic variation within the domesticated and wild genotypes contributed 3.8-fold more variation in resistance than domestication alone (Table 1).~~ ~~While we did observe the expected decreased resistance in domesticated tomato, domestication was a minor player in controlling lesion size variation, with most of the plant genetic signature coming from variation within both the wild and domesticated tomato species.~~ Removing the two domestication-associated isolates (Fd2, Rose) from our population did not eliminate the effect of tomato domestication on lesion size, ~~as Domestication still accounted for 1.2% of genetic variation, with a significant effect on lesion size~~as it was still significant and *B. cinerea* was still more virulent on domesticated tomato by 17% (Supplemental Table 1). To test how this mild domestication effect might be sensitive to shifts in the collection of tomato genotypes, we used the ~~bootstrapping of domestication~~same bootstraps from above for the full model ~~from above~~. Our observed domestication effect was in the top 80th percentile across all bootstraps, suggesting that ~~it~~while the domestication effect is small, it is relatively stable in response to shifts in the genotypes. However, a larger sample of *S. lycopersicum* and *S. pimpinellifolium* genotypes would be needed to develop a more precise estimate of any domestication effect on lesion size.

In addition to altering trait means, domestication commonly decreases genetic variation in comparison to wild germplasm due to bottlenecks, including for tomato (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Bai and Lindhout 2007). We would expect this decreased genetic variation to limit phenotypic variation, including disease phenotypes. Interestingly in this tomato population, we did not observe reduced variation in lesion size in the ~~wild~~domesticated tomato. ~~Rather, the domesticated tomato genotypes had a wider range of average lesion size than wild genotypes; the 90th percentile range (95th percentile to 5th percentile) spanned 2.03 cm² lesion size variation on domesticated tomato (standard deviation = 0.68 cm²) versus 1.76 cm² variation on wild tomato (standard deviation = 0.58 cm²). Additionally, the~~The wild and domesticated tomato genotypes showed ~~statistically~~ similar variation in resistance (F-test, $F_{96,96}=1.39$, p=0.11) (Figure 3, Supplemental Figure 2~~1~~). Overall, there is a slight

468 domestication impact on average resistance to *B. cinerea*, ~~but~~ and no evidence of a
469 phenotypic bottleneck due to domestication. This suggests that in the tomato-*B. cinerea*
470 pathosystem, domestication is not a major part of the variation.

471

472 **Quantitative Genetics of Pathogen Virulence on Tomato**

473   Genetic variation within *B. cinerea* had a large effect on virulence on tomato and
474 showed some evidence for interact~~ioned~~ with tomato domestication (Table 1). This
475 suggests that there is genetic variation within the pathogen, in which some alleles
476 enhance, and other alleles decrease virulence depending upon the plant's genotype. To
477 identify variable pathogen genes controlling differential virulence across plant
478 genotypes, we conducted GWA mapping analysis within the pathogen ~~by two~~
479 ~~approaches,~~ using 272,672 SNPs compared to the *B. cinerea* T4 reference genome
480 (Supplemental Figure 1) (Atwell, Corwin et al. 2018). Due to the large effect of plant
481 genotype on resistance to *B. cinerea*, we performed GWA using ~~the~~ model-corrected
482 least-squared mean virulence measured on each tomato genotype as separate traits.
483 We ~~first~~ used a ridge-regression approach (bigRR) ~~in combination with 272,672 SNPs~~
484 ~~from *B. cinerea* compared to the T4 reference genome~~ to estimate the phenotypic
485 effects across the genome ~~(Shen, Alam et al. 2013, Corwin, Copeland et al. 2016,~~
486 ~~Corwin, Subedy et al. 2016, Francisco, Joseph et al. 2016)~~ (Shen, Alam et al. 2013,
487 Corwin, Copeland et al. 2016, Corwin, Subedy et al. 2016, Francisco, Joseph et al.
488 2016, Atwell, Corwin et al. 2018). ~~To verify these patterns, we also implemented a~~
489 ~~Genome-wide Efficient Mixed-Model Association (GEMMA) analysis with a centered~~
490 ~~relatedness matrix to control for the effects of population structure (Zhou and Stephens~~
491 ~~2012). In GEMMA, we included 237,878 SNPs from *B. cinerea* compared to the B05.10~~
492 ~~reference genome.~~ To determine significance of SNP effects under ~~both~~ GWA ~~methods~~,
493 we permuted phenotypes 1000 times to calculate 95, 99, and 99.9% effect size
494 thresholds within each plant host. At~~Under~~ 1000~~x~~ permutations, the 99.9% threshold is
495 imprecise, but we included this approximate threshold to ~~more conservatively~~ identify
496 conservativel~~y~~ SNP associations. ~~Under both methods,~~ GWA analysis showed that the
497 genetic basis of *B. cinerea* virulence on tomato is highly polygenic. Consistent with a
498 polygenic structure of this trait in the pathogen, ~~neither method of GWA identified~~GWA

did not identify large-effect SNPs (Figure 4). The number of significant *B. cinerea* virulence SNPs identified by this ridge-regression approach (bigRR) ~~identified from~~varied by plant accession, from 1,284 to 25,421 SNPs on ~~within *B. cinerea* that were significantly associated with altered virulence on~~ the 12 different host genotypes (significance was determined by the SNP effect size estimate exceeding the 99% 1000-permutation threshold). ~~The model accounting for population structure (GEMMA) confirmed our finding of a highly polygenic nature of lesion size in the pathogen (Supplemental Figure 3), with 2,530 to 8,221 SNPs significantly associated with virulence at the 99% threshold, and 288 to 1,361 SNPs at the 99.9% threshold (significance was determined using an empirically determined 1000-permutation threshold).~~

At the SNP level, fewer loci contribute to virulence across all host~~s~~ genotypes. We found five *B. cinerea* SNPs significantly linked to altered lesion size on all 12 tomato accessions (Figure 4b). 215 SNPs were called in at least ten hosts, and 3~~.~~,300~~k~~ SNPs were called in at least half of the hosts while 27% (46,000) of the significant SNPs were linked to virulence on only a single host tomato genotype. These levels of overlap exceed the expected overlap due to random chance (Figure 5a). While only a small subset of these *B. cinerea* SNPs were linked to virulence on all the tomato genotypes, we obtained better overlap across host genotypes by focusing on gene windows.

To focus on the small-effect genes linked to *B. cinerea* virulence, we classified a gene as significantly associated if there was 1 SNP linked to a trait using a 2kbp window surrounding the start and stop codon for a given gene. This analysis identified 14 genes linked to differential virulence in all 12 tomato accessions by bigRR (Figure 5b, Supplemental Data Set 2 ~~a~~e), as some SNPs within a gene had accession-specific phenotypes (significant in <12 tomato accessions). A further 1045 genes were linked to differential virulence on 7 to 11 of the tomato accessions by bigRR (Figure 5b, Supplemental Data Set 2a ~~e~~). ~~At the 99.9% SNP significance threshold, GEMMA identified 23 genes across 7 to 9 of the tomato accessions (Supplemental Figure 4).~~

Of the 14 genes with SNPs significantly associated with *B. cinerea* virulence on all tomato genotypes by bigRR, most have not been formally linked to pathogen virulence. However, SNPs within a pectinesterase gene (BcT4_6001, Bcin14g00870)

530   were associated to virulence across 11 tomato accessions. Pectinesterases are key

531   enzymes for attacking the host cell wall, suggesting that variation in this pectinesterase

532   locus and the other loci may influence pathogen virulence across all the tomato

533   genotypes (Valette-Collet, Cimerman et al. 2003). Therefore, as an example of a

534   virulence gene identified by our GWA methods, we looked for evidence of multiple

535   haplotypes in this locus linked to virulence by visualizing the SNP effects across the

536   pectinesterase gene. We plotted the effect sizes for all SNPs in this gene and

537   investigated the linkage disequilibrium amongst these SNPs (Figure 6). This showed

538   that the effect of SNPs across this gene vary in effect direction depending on tomato

539   host genotype (Figure 6a). We identified two haplotype blocks contributing to the

540   association of this gene to the virulence phenotype (Figure 6b). One block is associated

541   with SNPs in the 5' untranslated region in SNPs 5-11, and the second block is SNPs

542   that span the entirety of the gene in SNPs 13-26. Interestingly, there are only two SNPs

543   in the open reading frame of the associated gene (Figure 6). This suggests that the

544   major variation surrounding this locus is controlling the regulatory motifs for this

545   pectinesterase. Thus, there is significant genetic variation in *B. cinerea* virulence that is

546   dependent upon the host's genetic background. This suggests that the pathogen relies

547   on polygenic small effect loci, potentially allowing selection to customize virulence on

548   the different tomato hosts.

549   ~~To identify genes consistently associated with *B. cinerea* virulence on tomato~~

550   ~~across GWA methods, we examined the gene overlap between significant associations~~

551   ~~identified by GEMMA on the B05.10 genome and bigRR on the T4 genome. We~~

552   ~~conservatively identified genes within 2kb of significant SNPs at the 99% permutation~~

553   ~~threshold for bigRR, and at the 99.9% permutation threshold for GEMMA. Among these,~~

554   ~~263 genes were linked to at least two plant genotypes by both methods (Supplemental~~

555   ~~Data 2 a). These genes include transporters and enzymes that can be important for~~

556   ~~Botrytis toxin production and/or detoxification of plant defense compounds and are key~~

557   ~~to virulence. Other known and predicted pathogen virulence functions were largely not~~

558   ~~identified (Supplemental Data 2 a, c).~~

559   ~~At the SNP level, fewer loci contribute to virulence across all hosts and both~~

560   ~~GWA methods. We found five *B. cinerea* SNPs significantly linked to altered lesion size~~

561 on all 12 tomato accessions using the bigRR analysis (Figure 4b). 215 SNPs were

562 called in at least ten hosts, and 3.3k SNPs were called in at least half of the hosts while

563 27% (46,000) of the significant SNPs were linked to virulence on only a single host

564 tomato genotype. These levels of overlap exceed the expected overlap due to random

565 chance (Figure 5a). GEMMA analysis also found significant SNP overlap between hosts

566 at the 99% permutation threshold, with 89 SNPs in at least ten hosts, 859 SNPs in at

567 least half of the hosts, and 63% (19,270) of significant SNPs unique to a single host.

568 SNP calling between hosts was lower for GEMMA at the 99.9% permutation threshold,

569 with 78% of significant SNPs (4269) in a single host, and 38 SNPs significant across at

570 least half of the hosts (Supplemental Figure 4 a). While only a small subset of these *B.*

571 *cinerea* SNPs were linked to virulence on all the tomato genotypes, we obtained better

572 overlap across host genotypes by focusing on gene windows.

573

574 **Quantitative Genetics of Pathogen Response to Tomato Domestication**

575 The identification of two isolates that ~~distinctly respond~~differed on wild and

576 domesticated tomato ~~to tomato domestication suggests that there is~~ indicated that there

577 may be some natural genetic variation in *B. cinerea* ~~that is affected by tomato~~

578 ~~domestication~~linked to this phenotypic variation. To directly map *B. cinerea* genes that

579 control differential virulence on wild versus domesticated tomatoes, we used the least-

580 squared mean virulence of each isolate across all wild and all domesticated tomato

581 genotypes as two traits. We also calculated a domestication sensitivity trait; the relative

582 difference in lesion size for each isolate between domesticated and wild hosts. Using

583 these three traits, we conducted bigRR GWA within *B. cinerea* to map genes in the

584 pathogen that respond to domestication shifts in the plant. Using the mean lesion area

585 of the *B. cinerea* isolates on the wild or domesticated tomato hosts identified a complex,

586 highly polygenic pattern of significant SNPs, similar to the individual tomato accessions

587 (Figure 4, Figure 7). ~~This~~ The significant SNP sets had a high degree of overlap

588 between the wild phenotype and domesticated phenotype. In contrast, ~~the~~

589 Domestication Sensitivity ~~trait~~ identified a ~~much~~ more limited set of SNPs ~~that had~~with

590 less overlap ~~with~~ to the mean lesion area on either Domesticated or Wild tomato (Figure

591 7). GWA of these domestication traits by GEMMA identified similar patterns of polygenic

~~structure, high overlap between SNPs and genes on wild or domesticated tomato hosts,~~ ~~and rare overlap with Domestication Sensitivity (Supplemental Figure 5).~~ To ~~begin~~ ~~querying~~query the underlying gene functions for these ~~various~~ *B. cinerea* loci, we called genes as significant if there was one SNP within 2kb of the gene (Figure 7c). ~~We also~~ ~~examined the genes associated with these domestication virulence traits found by both~~ ~~bigRR and GEMMA. This overlap identified 200 unique genes including several~~ ~~transporters and enzymes, with few predicted virulence genes (Supplemental Data 2 b).~~ ~~One gene from this overlap list (Bcin01g05800) contains TPR repeats, which are~~ ~~common in bacterial virulence proteins (Cerveny, Straskova et al. 2013) and are among~~ ~~the proteins secreted by the plant pathogen *Ustilago maydis* (Lo Presti, López Díaz et~~ ~~al. 2016).~~ Using all 1251 genes linked to domestication traits by bigRR for a functional enrichment analysis found only 22 significantly overrepresented biological functions (Fisher exact test, p<0.05, Supplemental Data Set 2~~b~~f) when compared to the whole-genome T4 gene annotation. ~~We also examined functional enrichment for the genes~~ ~~associated with domestication traits by both GEMMA and bigRR. We found 41~~ ~~significantly overrepresented biological functions (Supplemental Data 2 d). In both~~ ~~datasets, the~~ The enrichments were largely surrounding enzyme and transport functions, which are known to be key components of how the pathogen produces toxic metabolites and conversely detoxifies plant defense compounds. Thus, there is an apparent subset of *B. cinerea* genes that may be specific to the genetic changes that occurred in tomato during domestication. Further work is needed to assess if and how variation in these genes may link to altered virulence on domesticated and wild tomatoes.

Discussion

The genetics of plant resistance to generalist pathogens are mostly quantitative, depend upon pathogen isolate, and rely on genetic variation in both signal perception and direct defense genes (Kover and Schaal 2002, Parlevliet 2002, Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Tiffin and Moeller 2006, Rowe and Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016, Zhang, Corwin et al. 2017). Previous studies ~~on~~ of tomato resistance to *B. cinerea* have

found a quantitative genetic architecture that varies between domesticated and wild tomato species, with higher resistance in the wild species (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002, Guimaraes, Chetelat et al. 2004, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Finkers, Bai et al. 2008). However, it was not known how the choice of *B. cinerea* isolate may change this plant-pathogen interaction. To address these questions, we used genetic variation in wild and domesticated tomato accessions in conjunction with a population of *B. cinerea* isolates. ~~This also allowed us to test how domestication variation within tomato influenced the interaction at the level of the pathogen population and individual genes in the pathogen.~~ *B. cinerea* virulence on tomato, as measured by lesion size, was significantly affected by pathogen isolate, host genotype, and domestication status (Table 1). Pathogen isolate and tomato genotype were the strongest determinants of the interaction with only ~~Tomato domestication led to~~ a slight but significant decrease in resistance to the pathogen associated with domestication. ~~but critically~~Equally, there was no evidence of a domestication bottleneck, with similar variance in resistance between the wild and domesticated tomato accessions (Table 1, Figure 2). There was also little evidence in this *B. cinerea* population for specialization to tomato, supporting the hypothesis that *B. cinerea* is a generalist at the isolate and species level (Figure 1 c-h) (Giraud, Fortini et al. 1999, Martinez, Blancard et al. 2003, Ma and Michailides 2005). GWA mapping within the pathogen showed that the genetics underlying *B. cinerea* virulence on tomato are highly quantitative and vary across tomato genotypes and domestication status (Figure 5, Figure 7). This analysis identified a small subset of pathogen genes whose variation contributes to differential virulence on most of the hosts tested, and a set of pathogen genes whose variation is responsive to tomato domestication (Supplemental Data Set 2 ~~b, d, f~~b). ~~We also identified a conservative subset of genes whose association to differential Botrytis cinerea virulence is consistent across GWA methods and reference genomes (Supplemental Data 2 a, b, c, d).~~

Domestication and altered pathogen virulence genetics

~~These results provide evidence of a mild tomato domestication effect on resistance to the generalist pathogen, *B. cinerea*. We measured an 18% increase in~~

susceptibility across domesticated varieties, but this represents explains less than 1% of the a small portion of the total variance of *B. cinerea* lesion size on tomato (Table 1a). As such, domestication status alone is a poor predictor of a specific tomato host's resistance to infection by *B. cinerea*. This suggests that while tomato domestication does affect this plant-pathogen interaction, it is not the primary factor defining the measured trait. Functional plant defenses within both the domesticated and wild tomato accessions may act to buffer variation in *B. cinerea* virulence, as has been shown for *A. thaliana* (Zhang, Corwin et al. 2017). The effect of tomato domestication varied across the *B. cinerea* isolates, with specific loci linked to differential virulence across wild and domesticated tomatoes (Figure 1 c-h, Figure 7, Supplemental Data Set 3, Supplemental Data Set 4)). If a study relies on one or a few isolates, it could obtain a falsely high or falsely low estimation of how host domestication influences pathogen resistance. This shows theAs such, future studies need to utilize a population of *B. cinerea* to understand the factors contributing to *B. cinerea* virulence and how this is altered by crop domestication.

In biotrophic pathogens, host domestication has decreased the diversity of resistance alleles because they are lost in the domestication bottleneck as found for specialist pathogens (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Hyten, Song et al. 2006, Chaudhary 2013). Surprisingly, we did not find evidence for a domestication bottleneck in the phenotypic resistance to *B. cinerea* (Figure 2, Figure 3). This is in contrast to genomic studies that explicitly show a genotypic bottleneck within tomato domestication (Miller and Tanksley 1990, Koenig, Jiménez-Gómez et al. 2013). Previous work in *A. thaliana* with these isolates has shown that if plant defenses such as jasmonic acid and salicylic acid signaling are non-functional, there is increased variation in *B. cinerea* virulence (Zhang, Corwin et al. 2017). Conversely, if major plant defenseThus, if these pathways such as jasmonic acid and salicylic acid lost functionality inhad large effect differences between wild and domesticated tomato tomato domestication, we would expect to see a wider range of *B. cinerea* virulence phenotypes in domesticated tomato (Zhang, Corwin et al. 2017). HoweverThe similarity in the variance suggests that any differences we are seeing are not caused by large effect changes that abolish or greatly diminish specific defense signaling networks , we

685 ~~also do not find evidence of phenotypic plasticity in domesticated tomato resistance to~~

686 ~~*B. cinerea*~~ (Figure 2 ~~, Figure~~and 3). ~~This suggests that at least for this generalist~~

687 ~~pathogen, the genetic bottleneck of tomato domestication has not imparted a~~

688 ~~phenotypic bottleneck. One possible explanation is that resistance to this pathogen is so~~

689 ~~polygenic in the plant that our experiment is not sufficiently large to pick up any genetic~~

690 ~~bottleneck effect using phenotypic variance.~~ These patterns, of mild decrease in

691 resistance to *B. cinerea* due to plant domestication, and within-species plant variation

692 exceeding the contribution of domestication itself, may be unique to interactions

693 between *B. cinerea* and tomato, or more general. ~~It remains to be seen if these patterns~~

694 ~~hold for *B. cinerea* on its other host plants.~~ It is unclear whether ~~domestication~~ this

695 pattern is unique to tomato~~has a universal effect on plant resistance to *B. cinerea*~~, or if

696 each domestication event is unique.

697

Polygenic quantitative virulence and breeding complications

699 Our results indicate a highly polygenic basis of quantitative virulence of the

700 generalist *B. cinerea* on tomato~~.~~ similar to the~~Previous studies have found a~~ highly

701 polygenic basis ~~of *B. cinerea* quantitative virulence~~ on the host side of the interaction

702 (Zhang, Corwin et al. 2017). The variation in lesion size is linked to numerous *B. cinerea*

703 SNPs, each with small effect sizes (Figure 4a). Importantly, the tomato host accession

704 greatly influenced which *B. cinerea* loci were significantly associated to lesion size

705 (Figure 5). Thus, it possible that there is specialization at the gene level, in which

706 different alleles within the pathogen link to differential virulence on specific host

707 genotypes (Giraud, Fortini et al. 1999, Rowe and Kliebenstein 2007, Blanco-Ulate,

708 Morales-Cruz et al. 2014). This polygenic architecture of virulence is ~~distinctly~~ different

709 from virulence architecture in specialist pathogens that often have one or a few large

710 effect genes that control virulence (Keen 1992, De Feyter, Yang et al. 1993,

711 Abramovitch and Martin 2004, Boyd, Ridout et al. 2013, Vleeshouwers and Oliver

712 2014). Further studies are needed to compare how the host plant species may affect

713 this image of genetic variation in virulence.

714 These results indicate particular challenges for breeding durable resistance to *B.*

715 *cinerea*, and possibly other generalist pathogens. The highly polygenic variation in

716 virulence, combined with genomic sequencing showing that this pathogen is an inter-

717 breeding population, suggests that the pathogen is actively blending a large collection of

718 polymorphic virulence loci ~~(Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012,~~

719 ~~Atwell, Corwin et al. 2015)~~ (Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012,

720 Atwell, Corwin et al. 2015, Atwell, Corwin et al. 2018). Thus, it is ~~not~~ insufficient to breed

721 crop resistance against a single isolate of *B. cinerea*, as this resistance mechanism

722 would likely be rapidly overcome by new genotypes within the field population of *B.*

723 *cinerea*. In contrast, it is likely necessary to breed resistance using a population of the

724 pathogen, and to focus on plant loci that target entire virulence pathways or

725 mechanisms. The results in this study indicate that the specific genetics of the plant

726 host, the host's general domestication status, and the specific genetics of the pathogen

727 isolate will all combine to affect how the estimated breeding value inferred from any

728 experiment will translate to a field application (Table 1). As such, utilizing a single or

729 even a few pathogen isolates to guide resistance breeding in plants is unlikely to

730 translate to durable resistance against *B. cinerea* as a species. Further, the lack of

731 evidence for a domestication bottleneck on tomato resistance to B. *cinerea* suggests

732 that, at least for tomato, allelic variation in this generalist pathogen is sufficient to

733 overcome introgression of wild resistance genes or alleles into the domesticated crop.

734   This study examined the contributions of host and pathogen natural genetic

735 variation to the quantitative interaction in the tomato-*B. cinerea* pathosystem. ~~In~~

736 ~~addition, the study explicitly tested the effects of tomato domestication on this~~

737 ~~pathosystem.~~ *B. cinerea* has a highly quantitative genetic basis of virulence on tomato,

738 which is dominated by pathogen effects but also sensitive to genetic variation linked to

739 tomato domestication. Future studies are necessary to test if this pattern of

740 domestication responses in tomato is similar to patterns in other crops. Because this

741 population of *B. cinerea* can infect a wide range of hosts, it will be possible to directly

742 conduct this study. By extending future work to additional domestication events, it may

743 be possible to test whether independent crop domestication events have a consistent

744 underlying genetic signal of *B. cinerea* adaptation to plant domestication.

745

746 **Methods**

Tomato genetic resources

We obtained seeds for 12 selected tomato genotypes in consultation with the UC Davis Tomato Genetics Resource Center. These include a diverse sample of 6 genotypes of domesticated tomato's closest wild relative (*S. pimpinellifolium*) sampling across its major geographic regions (Peru, Ecuador) and 6 heritage and modern varieties of *S. lycopersicum*, focusing on mid- to late-20th century improved varieties (Lin, Zhu et al. 2014, Blanca, Montero-Pau et al. 2015). While genetic data is not available for all of our *S. pimpinellifolium* accessions, 9 of the 12 accessions have been genotyped and span the mappable diversity in domesticated tomato and its close relatives (Sim, Durstewitz et al. 2012) (Supplemental Figure 21). We bulked all genotypes in long-day (16h photoperiod) greenhouse conditions at UC Davis in fall 2014. We grew plants under metal-halide lamps using day/night temperatures at 25°C/18°C in 4" pots filled with standard potting soil (Sunshine mix #1, Sun Gro Horticulture). Plants were watered once daily and pruned and staked to maintain upright growth. Fruits were collected at maturity and stored at 4°C in dry paper bags until seed cleaning. To clean the seeds, we incubated seeds and locule contents at 24°C in 1% protease solution (Rapidase C80 Max) for 2h, then rinsed them in deionized water and air-dried. We then stored seeds in a cool, dry, dark location until use.

To grow plants for detached leaf assays, we bleach-sterilized all seeds and germinated them on paper in the growth chamber using flats covered with humidity domes. At 7 days we transferred seedlings to soil (SunGro Horticulture, Agawam, MA) and grew all plants in growth chambers in 20°C, short-day (10h photoperiod) conditions with 180-190 uM light intensity and 60% RH. We bottom-watered with deionized water every two days for two weeks, and at week 3 watered every two days with added nutrient solution (0.5% N-P-K fertilizer in a 2-1- 2 ratio; Grow More 4-18-38). The plants were used for detached leaf assays 6 weeks after transferring seedlings to soil. Flowering in this system did not occur until minimally 9 weeks of age for any accession, and as such we were sampling midway between the juvenile/adult transition and any flowering time decision. This window has been successful to minimize any major ontogenetic effects on the pathogen/host interaction in other systems (Corwin, Copeland et al. 2016).

778

B. cinerea genetic resources

We utilized a previously described collection of *B. cinerea* isolates that were
isolated as single spores from natural infections of fruit and vegetable tissues collected
in California and internationally . (Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017)
(Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017, Atwell, Corwin et al. 2018). This
included five isolates obtained from natural infections of tomato. We maintained *B.*
cinerea isolates as conidial suspensions in 30% glycerol for long-term storage at -80°C.
For regrowth, we diluted spore solutions to 10% concentration in filter-sterilized 50%
grape juice, and then inoculated onto 39g/L potato dextrose agar (PDA) media. We
grew isolates at 25°C in 12h light and propagated every 2 weeks. Sequencing failed for
6 out of our 97 phenotyped isolates. For bigRR GWA mapping with the 91 isolates
genotyped in this study, we utilized a total of 272,672 SNPs against the *B. cinerea* T4
genome with minor allele frequency (MAF) 0.20 or greater, and less than 10% missing
calls across the isolates (SNP calls in at least 82/ 91 isolates) (Atwell, Corwin et al.
2018). For GEMMA mapping, we used 91 isolates with a total of 237,878 SNPs against
the *B. cinerea* B05.10 genome with MAF 0.20 or greater and less than 10% missing
calls. The overall SNP number was similar when using either reference genome.

796

Detached leaf assay

To study the effect of genetic variation in host and pathogen on lesion formation,
we infected detached leaves of 12 diverse tomato varieties with the above 97 *B. cinerea*
isolates. We used a randomized complete block design for a total of 6 replicates across
2 experiments. In each experiment, this included a total of 10 plants per genotype
randomized in 12 flats in 3 growth chambers. Each growth chamber block corresponded
with a replicate of the detached leaf assay, such that growth chamber and replicate
shared the same environmental block. At 6 weeks of age, we selected 5 leaves per
plant (expanded leaves from second true leaf or younger), and 2 leaflet pairs per leaf.
We randomized the order of leaves from each plant, and the leaflets were placed on 1%
phytoagar in planting flats, with humidity domes. Our inoculation protocol followed
previously described methods (Denby, Kumar et al. 2004, Kliebenstein, Rowe et al.

809 2005). Spores were collected from mature *B. cinerea* cultures grown on canned peach

810 plates and diluted to 10 spores/ µL in filter-sterilized 50% organic grape juice. Spores in

811 grape juice were maintained in 4°C refrigeration or on ice from the time of collection, to

812 inhibit germination prior to inoculation. The diluted spore suspensions were

813 homogenized by agitation continuously during the entire process of applying the spores

814 to all samples. This maintains the spores in the suspension and ensures even

815 application across samples, then 4µl droplets were placed onto the detached leaflets at

816 room temperature. The entire inoculation took approximately 2 hours of time per

817 experiment. Mock-inoculated control leaves were treated with 4µL of 50% organic grape

818 juice without spores. Digital photos were taken of all leaflets at 24, 48, and 72 hours

819 post inoculation and automated image analysis was used to measure lesion size.

820

821 **Automated Image Analysis**

822   Lesion area was digitally measured using the EBImage and CRImage packages

823 (Pau, Fuchs et al. 2010, Failmezger, Yuan et al. 2012) in the R statistical environment

824 (R Development Core Team 2008), as previously described (Corwin, Copeland et al.

825 2016, Corwin, Subedy et al. 2016). Leaflets were identified as objects with green hue,

826 and lesions were identified as low-saturation objects within leaves. Images masks were

827 generated for both the leaf and lesion, then manually refined by a technician to ensure

828 accurate object calling. The area of these leaves and lesions were then automatically

829 measured as pixels per lesion and converted to area using a 1 cm reference within each

830 image.

831

832 **Data analysis**

833   We analyzed lesion areas using a general linear models for the full experiment to

834 determine the contributions of plant and pathogen genotype, including the fixed effects

835 of isolate genotype, plant domestication (*S. lycopersicum* or *S. pimpinellifolium*), plant

836 genotype (which is nested within domestication status), experiment, and block (nested

837 within experiment) on lesion area, as well as their interactions ((R lme4 package;

838 (Bates, Maechler et al. 2015)) Two of our 97 isolates that did not have replication across

839 2 experiments were dropped at this stage of analysis. We used the following linear

840 models throughout our analyses.

841 Main mixed-effect model of lesion size variation
842 $Y = I + D/P + I:D + I:D/P + W_R/L/A + E_R + E_R:I$
843 Within-plant accession mixed-effect model of lesion size
844 $Y = I + W_R/L/A + E_R + E_R:I$
845 Within-isolate mixed-effect model of lesion size
846 $Y = D/P + E_R$

847

848 Where I represents fungal genotype (isolate), P represents plant genotype

849 (accession), D represents domestication status, E represents experiment, W represents

850 whole plant, L represents leaf, A represents leaflet position. Factors with the subscript R

851 are included in the analysis as random effects.

852 The significance of individual terms in the model did not change if experiment

853 and block were treated as random effects. Adding terms for individual plant, leaf, and

854 leaflet position did not significantly improve the full model, so they were omitted from

855 further analysis. This The within-plant accession model was used to calculate the

856 significance of each factor and to obtain the least-squared means of lesion size for each

857 *B. cinerea* isolate x tomato accession as well as for each *B. cinerea* isolate x

858 domesticated/ wild tomato. We also calculated a domestication sensitivity phenotype,

859 Sensitivity = (Domesticated lesion size – Wild lesion size) / Domesticated lesion size.

860 We bootstrapped assignment of plant accessions to domestication groups in

861 order to assess the robustness of our observed domestication effects. We randomly

862 drew three genotypes from the domesticated and wild groupings and assigned them to

863 a new pseudo-wild grouping. The other six genotypes were assigned as a pseudo-

864 domesticated grouping and the model was rerun. This bootstrapping was repeated 100

865 times with each representing a random draw. We used these to repeat the full model

866 and to repeat the individual isolate models, as a test of the robustness of the tomato

867 domestication effect.

868 Using tomato sequence data from the SolCAP diversity panel that contained 9 of

869 our 12 accessions, we determined pairwise genetic distances between our accessions

870 (Sim, Durstewitz et al. 2012). We calculated pairwise Euclidean distances between 426

871 wild and domesticated tomato accessions from Infinium SNP genotyping at 7,720 loci

872 using the R adegenet package (Jombart , Sim, Durstewitz et al. 2012). Clustering is by

873 R hclust (in the stats package) default UPGMA method (R Development Core Team

874 2008).

875   We used several methods to examine host specialization to tomato within *B.*

876 *cinerea*. First, we split our *B. cinerea* population into isolates collected from tomato

877 tissue vs. other hosts. We compared these groups by t-test for virulence on

878 domesticated tomato genotypes, wild tomato genotypes, or all tomato genotypes. Next,

879 we used a Wilcoxon signed-rank test to compare the rank order distribution of model-

880 adjusted lesion sizes across paired tomato genotypes. ~~To~~ Also, to examine host

881 specialization to tomato domestication within *B. cinerea*, we used a Wilcoxon signed-

882 rank test to compare the rank order of model-adjusted lesion sizes across all

883 domesticated vs. all wild tomato genotypes. Finally, we conducted single-isolate

884 ANOVAs with FDR correction on general linear models to identify isolates with a

885 significant response to plant genotype or domestication status.

886   The model means and Domestication Sensitivity were used as the phenotypic

887 input for GWA using bigRR, a heteroskedastic ridge regression method that

888 incorporates SNP-specific shrinkage (Shen, Alam et al. 2013). This approach has

889 previously had a high validation rate (Ober, Huang et al. 2015, Corwin, Copeland et al.

890 2016, Francisco, Joseph et al. 2016, Kooke, Kruijer et al. 2016). The *B. cinerea* bigRR

891 GWA used 272,672 SNPs at MAF 0.20 or greater and <10% missing SNP calls as

892 described above (Atwell, Corwin et al. 2018). Because bigRR provides an estimated

893 effect size, but not a p-value, significance was estimated using 1000 permutations to

894 determine effect significance at 95%, 99%, and (approximately) 99.9% thresholds

895 (Doerge and Churchill 1996, Shen, Alam et al. 2013, Corwin, Copeland et al. 2016).

896 SNPs were annotated by custom R scripts with gene transfer format file construction

897 from the T4 gene models for genomic DNA by linking the SNP to genes within a 2kbp

898 window (http://www.broadinstitute.org, (Staats and van Kan 2012)). Functional

899 annotations are based on the T4 gene models for genomic DNA

900 (http://www.broadinstitute.org, *B. cinerea*; (Staats and van Kan 2012)). Additional genes

901 of interest, based on a broad literature search of known virulence loci, were taken from

902 NCBI (https://www.ncbi.nlm.nih.gov/) and included by mapping sequence to the T4

903 reference using MUMmer v3.0 (Kurtz, Phillippy et al. 2004).

904   To predict expected overlap of significant SNPs across plant genotypes, we used

905 the average number of significant SNPs per each of the 12 plant genotypes (14,000

906 SNPs) and calculated expected overlap between those 12 lists using binomial

907 coefficients. ~~The *B. cinerea* GEMMA used 237,878 SNPs at MAF 0.20 or greater, and~~

908 ~~less than 10% missing SNP calls as described above. To determine significance of~~

909 ~~SNPs by GEMMA, we used 1000 permutations to determine p-value significance at the~~

910 ~~99%, and 99.9% thresholds (Doerge and Churchill 1996, Shen, Alam et al. 2013,~~

911 ~~Corwin, Copeland et al. 2016). SNPs were annotated using a custom R script linking the~~

912 ~~SNP to genes within a 2kbp window from the gene transfer format file construction from~~

913 ~~the B05.10 gene models for genomic DNA (Staats and van Kan 2012, Zerbino,~~

914 ~~Achuthan et al. 2017). A table of gene name translations across genome annotations~~

915 ~~was pulled from the gene overlap between the bigRR T4 annotation and GEMMA~~

916 ~~B05.10 annotation using a custom R script and gene name translations pulled from the~~

917 ~~INRA *Botrytis cinerea* Portal (Choquer, Fournier et al. 2007, Viaud, Adam-Blondon et al.~~

918 ~~2012).~~ Functional annotations of the ~~overlap~~ gene lists are based on the T4 gene

919 models for genomic DNA (http://www.broadinstitute.org, *B. cinerea*; (Staats and van

920 Kan 2012).

921

922 **Supplemental Data Files**

923 Supplemental Data Set 1. Mean ± SE of *B. cinerea* lesion size of all isolates across all

924 tomato accessions.

925 Supplemental Data Set 2. Gene and Function Annotation from ~~B05.10 and~~ T4 GWA

926 Results

927 Supplemental Data Set 3. Results of single-isolate ANOVA on mixed effect model

928 Supplemental Table 1. Results of ANOVA following removal of domestication-

929 associated isolates

930 Supplemental Figure 1. Allele frequency spectrum of *B. cinerea* SNPs.

931 Supplemental Figure 2~~1~~. Genetic distance between selected tomato accessions~~.~~

932
934 Supplemental Figure 4~~2~~. Rank order plot of *B. cinerea* lesion size on two tomato
935 genotypes~~.~~

936

937 ~~Supplemental Figure 3. GWA by GEMMA of *B. cinerea* lesion size on individual tomato~~
938 ~~genotypes.~~
939 ~~Supplemental Figure 4. Frequency of overlap in *B. cinerea* GEMMA GWA significance~~
940 ~~across tomato accessions.~~
941 ~~Supplemental Figure 5. GEMMA GWA analysis of domestication sensitivity in *B.*~~
942 ~~*cinerea.*~~

943

944
945 **Figure Legends**

946

947 **Figure 1. *Botrytis cinerea* x tomato diversity in detached leaf assay and digital**
948 **image analysis.** a) Individual tomato leaflets of 6 *S. lycopersicum* genotypes and 6 *S.*
949 *pimpinellifolium* genotypes are in randomized rows, spore droplets of individual *B.*
950 *cinerea* isolates are in randomized columns. Digital images are collected 72 hours post
951 inoculation. Single droplets of 40 *B. cinerea* spores are infected on randomized leaflets
952 using randomized isolates, and digital images are taken 72 hours post inoculation.
953 b) Digital masking of leaf and lesion is followed by automated measurement of area for
954 each lesion.
955 c-h) Variation in lesion size resulting of the interaction of *B. cinerea* and diverse tomato
956 genotypes.
957 c) Average lesion size of single *B. cinerea* isolates (line traces) across tomato host
958 genotypes grouped by domestication status.
959 d) Highlight of the common reference *B. cinerea* isolate B05.10.
960 e) Highlight of the ten highest-virulence isolates, as estimated by mean virulence across
961 all tomato genotypes.
962 f) Highlight of the ten most saprophytic, or low virulence, isolates, as estimated by mean
963 virulence across all genotypes.
964 g) Highlight of the five isolates collected from tomato tissue.
965 h) Highlight of the two isolates with significant domestication sensitivity.

966

967 **Figure 2. Distribution of tomato genotype susceptibility to infection with 97**
968 **genetically diverse *B. cinerea* isolates.**
969 Violin plots show the distribution of lesion size caused by B. cinerea isolates on each
970 tomato host genotype. Individual points are mean lesion size for each of the 97 different
971 isolate-host pairs. The boxes show the 75th percentile distribution, and the horizontal

972 line shows the mean resistance of the specific host genotype. The tomato genotypes
973 are grouped based on their status as wild or domesticated germplasm.
974
975 **Figure 3. Distribution of *B. cinerea* virulence by tomato domestication status.**
976 The violin plots show the mean virulence of each *B. cinerea* isolate on the tomato
977 genotypes, grouped as wild or domesticated germplasm. The domestication effect on
978 lesion size is significant (Table 1 ANOVA, p~~<2e 16~~=0.0006). The interaction plot
979 between the two violin plots connects the average lesion size of a single *B. cinerea*
980 isolate between the wild and domesticated germplasm.
981
982 **Figure 4. GWA of *B. cinerea* lesion size on individual tomato genotypes.**
983 Botrytis cinerea chromosomes are differentiated by shading, alternating light and dark
984 grey.
985 a) Manhattan plot of estimated SNP effect sizes from bigRR for *B. cinerea* lesion size
986 using a single tomato accession, LA2093. Permutation-derived thresholds are shown in
987 horizontal dashed lines.
988 b) The number of tomato accessions for which a *B. cinerea* SNP was significantly linked
989 to lesion development by bigRR using the 99% permutation threshold. Frequency is
990 number of phenotypes in which the SNP exceeds the threshold. Vertical dotted lines
991 identify regions with overlap between the top 100 large-effect SNPs for LA2093 and
992 significance across the majority (≥6) of tomato genotypes tested.
993
994 **Figure 5. Frequency of overlap in *B. cinerea* GWA significance across tomato**
995 **accessions.**
996 a) The frequency with which the *B. cinerea* SNPs significantly associate with lesion size
997 on the 12 tomato accessions using bigRR and the 99% permutation threshold. The
998 black line indicates the expected frequency of random overlap, given the number of
999 significant SNPs per plant genotype and size of total SNP set. The inset zooms in on
1000 the distribution for overlapping SNPs above 6 plant genotypes for easier visualization.
1001 There were no SNPs expected to overlap by random chance in the inset.
1002 b) The frequency with which *B. cinerea* genes significantly associated with lesion size
1003 on the 12 tomato accessions. Genes were called as significant if there was one
1004 significant SNP called at the 99% permutation threshold within the gene body, or within
1005 2kb of the gene body.
1006
1007 **Figure 6. Host specificity of significant SNPs linked to the gene BcT4_6001**
1008 **(Bcin14g00870).**
1009 a) SNPs with effects estimates above the 99% permutation threshold are colored by
1010 trait (plant ~~phenotype~~ accession in which the effect was estimated). Wild accessions are
1011 oranges (yellow to red shades) and domesticated accessions are blues (green to purple
1012 shades). BcT4_6001 (Bcin14g00870) is a pectinesterase gene linked to at least one
1013 significant SNP on all 12 of the tested tomato accessions by bigRR. The annotated
1014 exons are depicted as turquoise rectangles, with the start codon marked with an arrow
1015 indicating the direction of transcription. Red rectangles indicate corresponding linkage
1016 disequilibrium blocks from Figure 6b.

1017 b) Linkage disequilibrium plot, including all pairwise comparisons of SNPs in the 2kb
1018 region surrounding Bcin14g00870. The color scheme for each SNP pair is D'/LOD:
1019 white if LOD <2 and D' <1, bright red for LOD ≥2 and D'=1, intermediate shades for
1020 LOD≥2 and D'<1.
1021
1022 **Figure 7. GWA analysis of domestication sensitivity in *B. cinerea.***
1023 Domestication sensitivity of each isolate was estimated using the average virulence on
1024 the wild and domesticated tomato germplasm and using calculated Sensitivity. This was
1025 then utilized for GWA mapping by bigRR.
1026 a) The top 1000 SNPs that significantly affect lesion size across domesticated tomato,
1027 wild tomato or domestication sensitivity are shown. Significance is called as crossing
1028 the 99% permutation threshold.
1029 b) Venn diagram of overlapping SNPs identified as crossing the 99% permutation
1030 threshold for each trait.
1031 c) Venn diagram of overlapping genes identified as crossing the 99% permutation
1032 threshold for each trait. Genes were called as significant if there was one significant
1033 SNP within the gene body or within 2kb of the gene body.
1034
1035

1040

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