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11

12 **Title: Interactions of ~~T~~tomato and *Botrytis* genetic diversity: Parsing the**

13 **contributions of host differentiation, domestication and pathogen variation~~Crop~~**

14 **~~domestication T~~Domesticated tomato d~~Domestication and pathogen virulence:~~**

15 **~~Interactions of tomato host and Botrytis genetic diversity~~**

16

17 Short title: Interactions of tomato and Botrytis genetics

22

Abstract

Human selection during crop domestication alters numerous traits, including disease resistance. While the impact of these processes on specialist pathogens are well-studied, less is known about the interaction of crop variation and generalist pathogens. To study how standing genetic variation and domestication within a crop 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*. 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 affected by large effects of the host and pathogen's genotype, with a much smaller contribution of domestication. This pathogen collection also enables genome-wide association (GWA) mapping in *B. cinerea*. GWA in the pathogen showed that virulence is highly polygenic and involves a diversity of mechanisms. This suggests that breeding against this pathogen would need to utilize 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. This indicates that this generalist pathogen already has a large collection of allelic variation that must be considered when designing a breeding program.

> Commented [C1]: Missing a verb in here

Introduction

54

55   Plant disease is mediated by complex interactions among diverse host and

56 pathogen molecular pathways, and the disease outcome is the sum of host plant

57 susceptibility/resistance and pathogen virulence/sensitivity mechanisms. The specific

58 outcome of any interaction is highly dependent on the genetic variation within these

59 pathways in both the host and pathogen. Over time, mutation and selection have led to

60 distinct genetic architectures in the host and pathogen that are at least partly influenced

61 by the host range of the pathogen. Specialist pathogens are a major focus in plant

62 pathology; virulent on a narrow range of hosts, and often limited to a single species or

63 genus. Most known plant genes for resistance to specialist pathogens confer qualitative

64 resistance through innate immunity via large-effect loci that enable the recognition of the

65 pathogen (Dangl and Jones 2001, Jones and Dangl 2006, Dodds and Rathjen 2010,

66 Pieterse, Van der Does et al. 2012). These recognition signals can be conserved

67 pathogen patterns such as cell-wall polymers or flagellin, or alternatively, specific

68 virulence factors that block perception of the pathogen, and in turn are detected by plant

69 proteins that guard the signaling networks (Jones and Dangl 2006, Bittel and Robatzek

70 2007, Ferrari, Galletti et al. 2007, Boller and He 2009, Dodds and Rathjen 2010). The

71 evolution of large-effect qualitative loci has partly been driven by the narrow host range

72 for the pathogen that enhances co-evolution between host resistance genes and

73 pathogen virulence mechanisms.

74   In contrast to specialist pathogens, generalist pathogens are virulent across a

75 wide range of plant host species. Generalist pathogens potentially have less stringent

76 co-evolution to specific hosts and their accompanying resistance mechanisms, because

77 these pathogens can easily shift to new hosts in the environment. This allows generalist

78 pathogens to evade the rapid evolution of new resistance mechanisms within specific

79 hosts until they evolve to counter this new resistance. This niche-shifting ability may

80 partially explain the observation that most natural resistance to generalist pathogens is

81 highly polygenic, and the underlying plant genes for resistance are quantitative

82 (Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Rowe and

83 Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016). Plant

84 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

similarly hold for generalist pathogens and their domesticated hosts. However, there is less information about how crop host domestication affects disease caused by generalist pathogens, when the resistance to these pathogens is quantitative and polygenic rather than qualitative and monogenic. As such, there is a need to quantify the effect of domestication on a broad generalist pathogen in comparison to the rest of the crop's standing variation to test conduct a detailed analysis ofanalyze how domestication may alter the interaction of a plant with a broad generalist pathogen, and correspondingly, how and if how domestication influences the pathogen.

Botrytis cinerea provides a model generalist pathogen for studying quantitative interactions with plant hosts and underlying evolutionary processes. for this generalist in contrast to specialist pathogens. B. cinerea is a broad generalist pathogen that can infect most tested plants, from bryophytes to eudicots, and causes wide ranging pre- and post-harvest crop losses (Nicot and Baille 1996, Elad, Williamson et al. 2007, Fillinger and Elad 2015). Individual isolates of B. cinerea show the same broad host range (Deighton, Muckenschnabel et al. 2001, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Corwin, Subedy et al. 2016), (Deighton, Muckenschnabel et al. 2001, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Corwin, Subedy et al. 2016). This is in contrast to pathogens like Fusarium oxysporum where the species can infect diverse hosts, but each isolate is highly host specific (Katan 1999, Ormond, Thomas et al. 2010, Loxdale, Lushai et al. 2011, Barrett and Heil 2012). B. cinerea isolates display significant variation in virulence phenotypes, partly due to genetic variation in specific virulence mechanisms, like the production of the phytotoxins, botrydial and botcinic acid (Siewers, Viaud et al. 2005, Dalmais, Schumacher et al. 2011). This genetic variation also influences cell wall degrading enzymes and key regulators of virulence like VELVET that quantitatively control virulence on multiple host plants (Rowe and Kliebenstein 2007, Schumacher, Pradier et al. 2012). This genetic variation in diverseThis standing diversity in virulence mechanisms can contribute to the formation of quantitative differences in virulence between the isolates (ten Have, Mulder et al. 1998). The phenotypic variation is driven by a high level of sequence diversity spread across the genome (Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012). The polymorphism rate in B. cinerea

was measured as, 6.6 SNP/kb, which is more variable than most previously studied plant pathogens (1-2 SNP/kb in *Blumeria graminis*, 1.5 SNP/kb in *Melampsora larici-populina*, 5.5 SNP/kb in the compact genome of the obligate biotroph *Plasmodiophora brassicae*), and close to the genetic diversitythat found in the human pathogen *Mycobacterium tuberculosis* (2.9 to 6.2 SNP/kb) (Farhat, Shapiro et al. 2013, Hacquard, Kracher et al. 2013, Wicker, Oberhaensli et al. 2013, Persoons, Morin et al. 2014, Atwell, Corwin et al. 2015, Desjardins, Cohen et al. 2016, Power, Parkhill et al. 2017). Higher polymorphism rates are reported for the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici*, from a small non-random sample of isolates (12.3 SNP/kb) (Upadhyaya, Garnica et al. 2014). In addition to SNP diversity, the genomic sequencing showed that *B. cinerea* has a high level of recombination and genomic admixture, as if it were a randomly intermating population (Supplemental Figure 1). As such, a collection of *B. cinerea* isolates contains genetic variation in a wide range of virulence mechanisms, offering the potential to challenge the host with a blend of diverse virulence mechanisms. to identify the pathogen variation controlling quantitative virulence (Corwin, Subedy et al. 2016, Bartoli and Roux 2017, Fordyce, Soltis et al. 2018). This can potentially identify the pathogen variation controlling quantitative virulence, even in non-model plant systems (Bartoli and Roux 2017).

A model pathosystem for studying quantitative host-pathogen interactions during domestication is the tomato-*B. cinerea* system, where the pathogen causes crop loss due to both pre- and post-harvest infection (Dean, Van Kan et al. 2012, Hahn 2014, Romanazzi and Droby 2016). Resistance to *B. cinerea* is a quantitative trait in tomato as with most other species, with identified tomato QTLs each explaining up to 15% of phenotypic variation for lesion size on stems (Dıaz, ten Have et al. 2002, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016). Tomato is also a key model system to study how domestication influences plant physiology and resistance, including alterations in the circadian clock (Tanksley 2004, Bai and Lindhout 2007, Panthee and Chen 2010, Bergougnoux 2014, Müller, Wijnen et al. 2016), which can modulate resistance to *B. cinerea* (Sauerbrunn and Schlaich 2004, Weyman, Pan et al. 2006, Bhardwaj, Meier et al. 2011, Hevia, Canessa et al. 2015). This suggests that host plant domestication

6

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

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

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

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

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

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

184   pathosystem.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

199   2015). In this study, we asked whether *B. cinerea* virulence was controlled by host

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

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

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

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

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

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

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

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

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

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

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

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

212 Gao, Liu et al. 2016, Talas, Kalih et al. 2016, Wu, Sakthikumar et al. 2017). Our

213 previously-sampled isolate collection includes genetic diversity across 272,672 SNPs

214 (Supplemental Figure 1) (Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017). To

215 ensure that genetic inference was independent of the GWA method or SNP diversity

216 reference, we repeated genetic analysis with two different association methods (bigRR

217 and GEMMA) using SNPs called in comparison to two published *B. cinerea* genomes

218 (T4 and B05.10). All methods converged on the same image of genetic architecture; We

219 found that the genetic architecture of virulence of *B. cinerea* is highly quantitative, with

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

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

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

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

224 generalist pathogen resistance, to inform breeding efforts.

225

226 **Results**

227 **Experimental Design**

228 To measure how tomato domestication genetic variation affects quantitative

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

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

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

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

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

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

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

236 2008, Müller, Wijnen et al. 2016)(Supplemental Figure 21). Our previously collected *B.*

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

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

239 Supersteak)(Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017). We infected all 97

B. cinerea isolates onto each of the 12 plant genotypes in 3-fold replication across 2 independent experiments in a randomized complete block design, giving 6 measurements per plant-pathogen combination, for a total of 3,276 lesions. Digital measurement of the area of the developing lesion provides a composite phenotype controlled by the interaction of host and pathogen genetics. This measurement of the plant-*B. cinerea* interaction has been used successfully in a number of molecular and quantitative genetic studies (Ferrari, Plotnikova et al. 2003, Denby, Kumar et al. 2004, Kliebenstein, Rowe et al. 2005, Ferrari, Galletti et al. 2007, Ten Have, van Berloo et al. 2007, AbuQamar, Chai et al. 2008, Rowe and Kliebenstein 2008, Liu, Hong et al. 2014). It should be noted that we are not focusing on MAMP or PAMP specific host/pathogen interactions with this study, we are instead allowing the identification of any mechanism that may influence the host/pathogen interaction including metabolism, development or any other unknown component. If there is genetic variation affecting the trait, and the trait influences the interaction of host and pathogen, it will be a component of the experiment. This fits with the recently developing view that growth, development and resistance in plants are highly integrated processes that may not be as distinct as once believed (Campos, Yoshida et al. 2016, Ballaré and Pierik 2017, Züst and Agrawal 2017, Izquierdo-Bueno, González-Rodríguez et al. 2018).

Lesion size (phenotypic) variation

We collected images of all lesions at 24, 48, and 72 hours post inoculation. At 24 hours, no visible lesions were present on the tomato leaves. At 48 hours, a thin ring of primary lesion became visible surrounding the location of the spore droplet, but no expansion was visible. At 72 hours significant lesion growth was visible, but no lesions had spread 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, ~~According~~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,

9

271 Corwin, Copeland et al. 2016, Fordyce, Soltis et al. 2018). And finally, previous work

272 has shown that Botrytis isolates display large variation in their unit biomass per ~~area~~

273 lesion area and as such growth in biomass is not the sole factor driving this

274 measure~~Lesion area is a common measure of the interaction of plant immunity and~~

275 ~~pathogen virulence, though pathogen biomass does not scale with lesion area~~ (Corwin,

276 Subedy et al. 2016) ~~(Rowe, Walley et al. 2010, Corwin, Copeland et al. 2016)~~ We

277 observed a mean lesion size of 0.67 cm^2 across the full experiment, with 0.94 CV

278 across the full isolate population on all tomato genotypes. Individual isolates were highly

279 variable in their lesion size across tomato genotypes (Figure 1 c-h), with mean lesion

280 size per isolate of 0.14 cm^2 to 1.29 cm^2, and individual isolate coefficient of variation

281 (CV) from 0.51 to 1.68 across all observations on all tomato genotypes (Supplemental

282 Data Set 1). A subset of these isolates is highly virulent on tomato (mean lesion size >

283 1.05 cm^2, Figure 1e), and a subset can be considered saprophytic (mean lesion size <

284 0.3 cm^2, Figure 1f). Lesion size of *B. cinerea* on tomato ~~was weakly~~showed a weak

285 positive ~~correlated~~correlation with lesion size on *A. thaliana* from previous studies; both on

286 domesticated tomato (r=0.247, p= 0.003) and on wild tomato (r=0.301, p= 0.016)

287 (Supplemental Figure 3)(Zhang, Corwin et al. 2017). This lack of correlation

288 ~~s~~Suggest~~sing~~ the presence of~~at there are~~ both shared and unique mechanisms of

289 quantitative virulence i~~o~~n the two species.

290

291

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

294   To measure the relative contribution of genetic diversity in the plant and the

295 pathogen to variation in the virulence/ susceptibility phenotype, we used a ~~multiple~~

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

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

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

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

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

301 lesion growth, ~~with pathogen isolate diversity explaining 3.5 fold~~4 more variance than

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

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

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

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

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

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

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

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

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

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

312

313 **Table 1. ANOVA results of the interaction between 12 tomato accessions and 95**
314 ***B. cinerea* isolates measured as lesion area.**
315 Results of general linear modelling of lesion area for 12 tomato accessions by 95 *B.*
316 *cinerea* isolates is shown (R lme4 package version 1.1-18-1;(Bates, Maechler et al.
317 2015)). Two of our 97 isolates did not have replication across 2 experiments, so they
318 were dropped at this stage of analysis. The terms are as follows; Isolate is the 95 *B.*
319 *cinerea* isolates, Domestication is wild tomato, *S. pimpinellifolium*, versus domesticated
320 tomato, *S. lycopersicum*, Plant is 12 tomato genotypes nested within their respective
321 domestication groupings, Experiment tests the random effect of 2 independent replicate
322 experiments. The nested random effects of whole plant sampled, leaf sampled, and
323 leaflet pair are included. In addition, interactions of these factors are tested (:). The
324 degrees of freedom and p-value are shown. For fixed effects, the type II sum of squares
325 and F-value are shown, and for random effects the likelihood ratio test statistic (LRT) is
326 shown.

327

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**	

328

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

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

331   domestication status.

332   **Pathogen Specialization to Source Host**

333   One evolutionary model of plant-generalist pathogen interactions suggests that

334   ~~generalist~~ pathogen isolates within ~~a~~ a generalist species may specialize for interaction

335   with specific hosts. Alternatively, generalist isolates may show no host specialization or

336   preference. Our collection of *B. cinerea* includes five isolates that may be adapted to

337   tomato, as they were collected from *S. lycopersicum*. To test if there is evidence for

338   specialization to the source host, we compared the virulence of the *B. cinerea* isolates

339   obtained from tomato to the broader pathogen population. For *B. cinerea* genotypes

340   isolated from tomato tissue vs. other hosts, there was no significant difference in lesion

341   size ~~on domesticated tomato (t-test; t=1.10, n = 97, p=0.33), wild tomato (t-test; t=1.09,~~

342   ~~n = 97, p=0.33) or~~ across all tomato genotypes (t-test; n = 97, p=0.14) (Figure 1g). In

343   fact, one isolate collected from tomato tissue (KGB1) was within the 10 least-virulent

344   isolates and another (Triple3) was within the 10 most-virulent isolates (Figure 1g). This

345   demonstrated significant genetic variation in virulence across the *B. cinerea* isolates,

346   and that this collection of *B. cinerea* isolates from tomato do not display a strong host-

347   specificity for tomato (Martinez, Blancard et al. 2003, Ma and Michailides 2005, Rowe

348   and Kliebenstein 2007, Samuel, Veloukas et al. 2012).

349

350   **Pathogen Specialization to Host Genotype**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

366　(~~38~~ 39 out of 66) of tomato accession pairs had significantly different ranking of the

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

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

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

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

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

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

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

374　to the tomato genetic variation.

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

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

377　(78/97, 80%) are more virulent on domesticated than wild tomato (Figure 3;

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

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

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

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

382　general population behavior, we used single-isolate ANOVAs ~~To~~ to ~~identify~~ test if any

383　specific~~the~~ pathogen genotypes ~~most~~ had a significant ~~sensitive to~~strongly associated

384　~~with domestication~~association with domestication. ~~, we conducted single-isolate~~

385　~~ANOVAs on~~These general linear models ~~including~~ included the fixed effects of plant,

386　domestication, and the random effect of experiment~~,~~. After adjusting for multiple testing,

387　this identified~~and found~~ two isolates (Fd2, Rose) with a significant effect of

388　domestication on lesion size (p < 0.05, FDR corrected) (Figure 1h), both of which are

389　more virulent on domesticated tomato (Supplemental Data Set 3). ~~These included one~~

390　~~of the highly virulent isolates (Fd2), and one of the largely saprophytic isolates (Rose),~~

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.

		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**	

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

408 on domesticated tomato, and a broader pattern of *B. cinerea* sensitivity to tomato

409 genetic variation.

410 To assess whether isolates could appear domestication-associated due to

411 random chance, we bootstrapped assignment of plant accessions to domestication

412 groups. Across the 100 bootstraps, we96 of the 100 bootstraps identified no isolates

413 with domestication sensitivity, and the other four bootstraps identified only identified 2

414 isolates showing significant domestication association (FDR <0.01) to domestication in

415 4% of the random datasets. Therefore, our individual isolate observations are in the

416 96th percentile. ThisWhile this is suggestive, suggests that a more precise estimate of

417 isolate x domestication interactions would require larger experiments using either more

418 replication or additional plant genotypes.

419

420 Domestication and Lesion Size Variation

421 Existing literature predominantly reports that crop domestication decreases plant

422 resistance to pathogens (Smale 1996, Rosenthal and Dirzo 1997, Couch, Fudal et al.

423 2005, Dwivedi, Upadhyaya et al. 2008, Stukenbrock and McDonald 2008). While we did

424 observe the expected decreased resistance (by 18%) in domesticated tomato (Figure 2

425 and 3, Table 1), domestication was a minor player in controlling lesion size variation,

426 with most of the plant genetic signature coming from variation within both the wild and

427 domesticated tomato species, contributing 12-fold more variation in resistance than

428 domestication alone (Table 1). In our analysis, we identified a significantly greater

429 (18%) resistance of wild tomato in comparison to domesticated tomato across the

430 population of *B. cinerea* isolates (Figure 2 and 3, Table 1). However, this domestication

431 effect was not the dominant source of variation, as genetic variation within the

432 domesticated and wild genotypes contributed 3.8 fold more variation in resistance than

433 domestication alone (Table 1). While we did observe the expected decreased

434 resistance in domesticated tomato, domestication was a minor player in controlling

435 lesion size variation, with most of the plant genetic signature coming from variation

436 within both the wild and domesticated tomato species. Removing the two domestication-

437 associated isolates (Fd2, Rose) from our population did not eliminate the effect of

438 tomato domestication on lesion size, as Domestication still accounted for 1.2% of

439 ~~genetic variation, with a significant effect on lesion size~~as it was still significant and *B.*

440 *cinerea* was still more virulent on domesticated tomato by 17% (Supplemental Data Set

441 4).

442 To test how this mild domestication effect might be sensitive to shifts in the

443 collection of tomato genotypes, we used the ~~bootstrapping of domestication~~same

444 bootstraps from above for the full model ~~from above~~. Our observed domestication effect

445 was in the top 80th percentile across all bootstraps, suggesting that ~~it~~while the

446 domestication effect is small, it is relatively stable in response to shifts in the genotypes.

447 However, a larger sample of *S. lycopersicum* and *S. pimpinellifolium* genotypes would

448 be needed to develop a more precise estimate of any domestication effect on lesion

449 size.

450 In addition to altering trait means, domestication commonly decreases genetic

451 variation in comparison to wild germplasm due to bottlenecks, including for tomato

452 (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Bai and Lindhout 2007). We

453 would expect this decreased genetic variation to limit phenotypic variation, including

454 disease phenotypes. Interestingly in this tomato population, we did not observe reduced

455 variation in lesion size in the ~~wild~~ domesticated tomato. ~~Rather, the domesticated~~

456 ~~tomato genotypes had a wider range of average lesion size than wild genotypes; the~~

457 ~~90th percentile range (95th percentile to 5th percentile) spanned 2.03 cm² lesion size~~

458 ~~variation on domesticated tomato (standard deviation = 0.68 cm²) versus 1.76 cm²~~

459 ~~variation on wild tomato (standard deviation = 0.58 cm²). Additionally, the~~ The wild and

460 domesticated tomato genotypes showed ~~statistically~~ similar variation in resistance (F-

461 test, $F_{96,96}=1.39$, p=0.11) (Figure 3, Supplemental Figure 2~~1~~). Overall, there is a slight

462 domestication impact on average resistance to *B. cinerea*, ~~but~~ and no evidence of a

463 phenotypic bottleneck due to domestication. This suggests that in the tomato-*B. cinerea*

464 pathosystem, domestication is not a major part of the variation.

465

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

467 Genetic variation within *B. cinerea* had a large effect on virulence on tomato and

468 showed some evidence for interact~~ioned~~ with tomato domestication (Table 1). This

469 suggests that there is genetic variation within the pathogen, in which some alleles

470 enhance, and other alleles decrease virulence depending upon the plant's genotype. To

471 identify variable pathogen genes controlling differential virulence across plant

472 genotypes, we conducted GWA mapping analysis within the pathogen by two

473 approaches, using 272,672 SNPs compared to the *B. cinerea* T4 reference genome

474 (Supplemental Figure 1). Due to the large effect of plant genotype on resistance to *B.*

475 *cinerea*, we performed GWA using the model-corrected least-squared mean virulence

476 measured on each tomato genotype as separate traits. We first used a ridge-regression

477 approach (bigRR) in combination with 272,672 SNPs from *B. cinerea* compared to the

478 T4 reference genome to estimate the phenotypic effects across the genome (Shen,

479 Alam et al. 2013, Corwin, Copeland et al. 2016, Corwin, Subedy et al. 2016, Francisco,

480 Joseph et al. 2016). To verify these patterns, we also implemented a Genome-wide

481 Efficient Mixed-Model Association (GEMMA) analysis with a centered relatedness

482 matrix to control for the effects of population structure (Zhou and Stephens 2012). In

483 GEMMA, we included 237,878 SNPs from *B. cinerea* compared to the B05.10 reference

484 genome. To determine significance of SNP effects under both GWA methods, we

485 permuted phenotypes 1000 times to calculate 95, 99, and 99.9% effect size thresholds

486 within each plant host. AtUnder 1000x permutations, the 99.9% threshold is imprecise,

487 but we included this approximate threshold to more conservatively identify

488 conservatively SNP associations. Under both methods, GWA analysis showed that the

489 genetic basis of *B. cinerea* virulence on tomato is highly polygenic. Consistent with a

490 polygenic structure of this trait in the pathogen, neither method of GWA identifiedGWA

491 did not identify large-effect SNPs (Figure 4). The number of significant *B. cinerea*

492 virulence SNPs identified by this ridge-regression approach (bigRR) identified

493 fromvaried by plant accession, from 1,284 to 25,421 SNPs on within *B. cinerea* that

494 were significantly associated with altered virulence on the 12 different host genotypes

495 (significance was determined by the SNP effect size estimate exceeding the 99% 1000-

496 permutation threshold). The model accounting for population structure (GEMMA)

497 confirmed our finding of a highly polygenic nature of lesion size in the pathogen

498 (Supplemental Figure 3), with 2,530 to 8,221 SNPs significantly associated with

499 virulence at the 99% threshold, and 288 to 1,361 SNPs at the 99.9% threshold

17

500 (significance was determined using an empirically determined 1000-permutation

501 threshold).

502 At the SNP level, fewer loci contribute to virulence across all hosts genotypes.

503 We found five *B. cinerea* SNPs significantly linked to altered lesion size on all 12 tomato

504 accessions (Figure 4b). 215 SNPs were called in at least ten hosts, and 3 ,300k SNPs

505 were called in at least half of the hosts while 27% (46,000) of the significant SNPs were

506 linked to virulence on only a single host tomato genotype. These levels of overlap

507 exceed the expected overlap due to random chance (Figure 5a). While only a small

508 subset of these *B. cinerea* SNPs were linked to virulence on all the tomato genotypes,

509 we obtained better overlap across host genotypes by focusing on gene windows.

510 To focus on the small-effect genes linked to *B. cinerea* virulence, we classified a

511 gene as significantly associated if there was 1 SNP linked to a trait using a 2kbp window

512 surrounding the start and stop codon for a given gene. This analysis identified 14 genes

513 linked to differential virulence in all 12 tomato accessions by bigRR (Figure 5b,

514 Supplemental Data Set 2 ae), as some SNPs within a gene had accession-specific

515 phenotypes (significant in <12 tomato accessions). A further 1045 genes were linked to

516 differential virulence on 7 to 11 of the tomato accessions by bigRR (Figure 5b,

517 Supplemental Data Set 2a e). At the 99.9% SNP significance threshold, GEMMA

518 identified 23 genes across 7 to 9 of the tomato accessions (Supplemental Figure 4).

519 Of the 14 genes with SNPs significantly associated with *B. cinerea* virulence on

520 all tomato genotypes by bigRR, most have not been formally linked to pathogen

521 virulence. However, SNPs within a pectinesterase gene (BcT4_6001, Bcin14g00870)

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

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

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

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

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

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

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

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

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

host genotype (Figure 6a). We identified two haplotype blocks contributing to the association of this gene to the virulence phenotype (Figure 6b). One block is associated with SNPs in the 5' untranslated region in SNPs 5-11, and the second block is SNPs that span the entirety of the gene in SNPs 13-26. Interestingly, there are only two SNPs in the open reading frame of the associated gene (Figure 6). This suggests that the major variation surrounding this locus is controlling the regulatory motifs for this pectinesterase. Thus, there is significant genetic variation in *B. cinerea* virulence that is dependent upon the host's genetic background. This suggests that the pathogen relies on polygenic small effect loci, potentially allowing selection to customize virulence on the different tomato hosts.

~~To identify genes consistently associated with *B. cinerea* virulence on tomato across GWA methods, we examined the gene overlap between significant associations identified by GEMMA on the B05.10 genome and bigRR on the T4 genome. We conservatively identified genes within 2kb of significant SNPs at the 99% permutation threshold for bigRR, and at the 99.9% permutation threshold for GEMMA. Among these, 263 genes were linked to at least two plant genotypes by both methods (Supplemental Data 2 a). These genes include transporters and enzymes that can be important for Botrytis toxin production and/or detoxification of plant defense compounds and are key to virulence. Other known and predicted pathogen virulence functions were largely not identified (Supplemental Data 2 a, c).~~

~~At the SNP level, fewer loci contribute to virulence across all hosts and both GWA methods. We found five *B. cinerea* SNPs significantly linked to altered lesion size on all 12 tomato accessions using the bigRR analysis (Figure 4b). 215 SNPs were called in at least ten hosts, and 3.3k 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). GEMMA analysis also found significant SNP overlap between hosts at the 99% permutation threshold, with 89 SNPs in at least ten hosts, 859 SNPs in at least half of the hosts, and 63% (19,270) of significant SNPs unique to a single host. SNP calling between hosts was lower for GEMMA at the 99.9% permutation threshold, with 78% of significant SNPs (4269) in a single host, and 38 SNPs significant across at~~

Quantitative Genetics of Pathogen Response to Tomato Domestication

The identification of two isolates that distinctly responddiffered on wild and domesticated tomato to tomato domestication suggests that there is indicated that there may be some natural genetic variation in *B. cinerea* that is affected by tomato domesticationlinked to this phenotypic variation. To directly map *B. cinerea* genes that control differential virulence on wild versus domesticated tomatoes, we used the least-squared mean virulence of each isolate across all wild and all domesticated tomato genotypes as two traits. We also calculated a domestication sensitivity trait; the relative difference in lesion size for each isolate between domesticated and wild hosts. Using these three traits, we conducted bigRR GWA within *B. cinerea* to map genes in the pathogen that respond to domestication shifts in the plant. Using the mean lesion area of the *B. cinerea* isolates on the wild or domesticated tomato hosts identified a complex, highly polygenic pattern of significant SNPs, similar to the individual tomato accessions (Figure 4, Figure 7). This The significant SNP sets had a high degree of overlap between the wild phenotype and domesticated phenotype. In contrast, the Domestication Sensitivity trait identified a much more limited set of SNPs that hadwith less overlap with to the mean lesion area on either Domesticated or Wild tomato (Figure 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 queryingquery 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 2b 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.

624 *B. cinerea* virulence on tomato, as measured by lesion size, was significantly affected

625 by pathogen isolate, host genotype, and domestication status (Table 1). Pathogen

626 isolate and tomato genotype were the strongest determinants of the interaction with only

627 ~~Tomato domestication led to~~ a slight but significant decrease in resistance to the

628 pathogen associated with domestication. ~~but critically~~Equally, there was no evidence of

629 a domestication bottleneck, with similar variance in resistance between the wild and

630 domesticated tomato accessions (Table 1, Figure 2). There was also little evidence in

631 this *B. cinerea* population for specialization to tomato, supporting the hypothesis that *B.*

632 *cinerea* is a generalist at the isolate and species level (Figure 1 c-h) (Giraud, Fortini et

633 al. 1999, Martinez, Blancard et al. 2003, Ma and Michailides 2005). GWA mapping

634 within the pathogen showed that the genetics underlying *B. cinerea* virulence on tomato

635 are highly quantitative and vary across tomato genotypes and domestication status

636 (Figure 5, Figure 7). This analysis identified a small subset of pathogen genes whose

637 variation contributes to differential virulence on most of the hosts tested, and a set of

638 pathogen genes whose variation is responsive to tomato domestication (Supplemental

639 Data Set 2 ~~b, d, f~~b). ~~We also identified a conservative subset of genes whose~~

640 ~~association to differential *Botrytis cinerea* virulence is consistent across GWA methods~~

641 ~~and reference genomes (Supplemental Data 2 a, b, c, d).~~

642

643 **Domestication and altered pathogen virulence genetics**

644 ~~These results provide evidence of a mild tomato domestication effect on~~

645 ~~resistance to the generalist pathogen, *B. cinerea*. We measured an 18% increase in~~

646 ~~susceptibility across domesticated varieties, but this represents~~ ~~explains less than 1% of~~

647 ~~the~~ ~~a small portion of the~~ ~~total variance of *B. cinerea* lesion size on tomato (Table 1a).~~

648 ~~As such, domestication status alone is a poor predictor of a specific tomato host's~~

649 ~~resistance to infection by *B. cinerea*. This suggests that while tomato domestication~~

650 ~~does affect this plant-pathogen interaction, it is not the primary factor defining the~~

651 ~~measured trait. Functional plant defenses within both the domesticated and wild tomato~~

652 ~~accessions may act to buffer variation in *B. cinerea* virulence, as has been shown for *A.*~~

653 ~~*thaliana* (Zhang, Corwin et al. 2017). The effect of tomato domestication varied across~~

654 ~~the *B. cinerea* isolates, with specific loci linked to differential virulence across wild and~~

655 ~~domesticated tomatoes (Figure 1 c-h, Figure 7, Supplemental Data Set 3, Supplemental~~

656 ~~Data Set 4)). If a study relies on one or a few isolates, it could obtain a falsely high or~~

657 ~~falsely low estimation of how host domestication influences pathogen resistance. This~~

658 ~~shows the~~As such, future studies ~~need to~~ utilize a population of *B. cinerea* ~~to~~

659 ~~understand the factors contributing to *B. cinerea* virulence and how this is altered by~~

660 ~~crop domestication.~~

661 In biotrophic pathogens, host domestication has decreased the diversity of

662 resistance alleles because they are lost in the domestication bottleneck as found for

663 specialist pathogens (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Hyten,

664 Song et al. 2006, Chaudhary 2013). Surprisingly, we did not find evidence for a

665 domestication bottleneck in the phenotypic resistance to *B. cinerea* (Figure 2, Figure 3).

666 This is in contrast to genomic studies that explicitly show a genotypic bottleneck within

667 tomato domestication (Miller and Tanksley 1990, Koenig, Jiménez-Gómez et al. 2013).

668 Previous work in *A. thaliana* with these isolates has shown that if plant defenses such

669 as jasmonic acid and salicylic acid signaling are non-functional, there is increased

670 variation in *B. cinerea* virulence (Zhang, Corwin et al. 2017). ~~Conversely, if major plant~~

671 ~~defense~~Thus, if these pathways ~~such as jasmonic acid and salicylic acid lost~~

672 ~~functionality in~~had large effect differences between wild and domestic~~ated~~ tomato

673 ~~tomato domestication,~~ we would expect to see a wider range of *B. cinerea* virulence

674 phenotypes in domesticated tomato (Zhang, Corwin et al. 2017). ~~However~~The similarity

675 in the variance suggests that any differences we are seeing are not caused by large

676 effect changes that abolish or greatly diminish specific defense signaling networks ~~, we~~

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

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

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

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

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

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

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

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

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

686 ~~hold for *B. cinerea* on its other host plants.~~ It is unclear whether ~~domestication~~ this
687 pattern is unique to tomato~~has a universal effect on plant resistance to *B. cinerea*~~, or if
688 each domestication event is unique.
689
690 **Polygenic quantitative virulence and breeding complications**
691   Our results indicate a highly polygenic basis of quantitative virulence of the
692 generalist *B. cinerea* on tomato~~.~~ similar to the~~Previous studies have found a~~ highly
693 polygenic basis ~~of *B. cinerea* quantitative virulence~~ on the host side of the interaction
694 (Zhang, Corwin et al. 2017)~~.~~ The variation in lesion size is linked to numerous *B. cinerea*
695 SNPs, each with small effect sizes (Figure 4a). Importantly, the tomato host accession
696 greatly influenced which *B. cinerea* loci were significantly associated to lesion size
697 (Figure 5). Thus, it possible that there is specialization at the gene level, in which
698 different alleles within the pathogen link to differential virulence on specific host
699 genotypes (Giraud, Fortini et al. 1999, Rowe and Kliebenstein 2007, Blanco-Ulate,
700 Morales-Cruz et al. 2014). This polygenic architecture of virulence is ~~distinctly~~ different
701 from virulence architecture in specialist pathogens that often have one or a few large
702 effect genes that control virulence (Keen 1992, De Feyter, Yang et al. 1993,
703 Abramovitch and Martin 2004, Boyd, Ridout et al. 2013, Vleeshouwers and Oliver
704 2014). Further studies are needed to compare how the host plant species may affect
705 this image of genetic variation in virulence.
706   These results indicate particular challenges for breeding durable resistance to *B.*
707 *cinerea*, and possibly other generalist pathogens. The highly polygenic variation in
708 virulence, combined with genomic sequencing showing that this pathogen is an inter-
709 breeding population, suggests that the pathogen is actively blending a large collection of
710 polymorphic virulence loci (Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012,
711 Atwell, Corwin et al. 2015). Thus, it is ~~not~~ insufficient to breed crop resistance against a
712 single isolate of *B. cinerea*, as this resistance mechanism would likely be rapidly
713 overcome by new genotypes within the field population of *B. cinerea*. In contrast, it is
714 likely necessary to breed resistance using a population of the pathogen, and to focus on
715 plant loci that target entire virulence pathways or mechanisms. The results in this study
716 indicate that the specific genetics of the plant host, the host's general domestication

24

status, and the specific genetics of the pathogen isolate will all combine to affect how the estimated breeding value inferred from any experiment will translate to a field application (Table 1). As such, utilizing a single or even a few pathogen isolates to guide resistance breeding in plants is unlikely to translate to durable resistance against *B. cinerea* as a species. Further, the lack of evidence for a domestication bottleneck on tomato resistance to B. *cinerea* suggests that, at least for tomato, allelic variation in this generalist pathogen is sufficient to overcome introgression of wild resistance genes or alleles into the domesticated crop.

This study examined the contributions of host and pathogen natural genetic variation to the quantitative interaction in the tomato-*B. cinerea* pathosystem. In addition, the study explicitly tested the effects of tomato domestication on this pathosystem. *B. cinerea* has a highly quantitative genetic basis of virulence on tomato, which is dominated by pathogen effects but also sensitive to genetic variation linked to tomato domestication. Future studies are necessary to test if this pattern of domestication responses in tomato is similar to patterns in other crops. Because this population of *B. cinerea* can infect a wide range of hosts, it will be possible to directly conduct this study. By extending future work to additional domestication events, it may be possible to test whether independent crop domestication events have a consistent underlying genetic signal of *B. cinerea* adaptation to plant domestication.

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

748　genotypes in long-day (16h photoperiod) greenhouse conditions at UC Davis in fall

749　2014. We grew plants under metal-halide lamps using day/night temperatures at

750　25°C/18°C in 4" pots filled with standard potting soil (Sunshine mix #1, Sun Gro

751　Horticulture). Plants were watered once daily and pruned and staked to maintain upright

752　growth. Fruits were collected at maturity and stored at 4°C in dry paper bags until seed

753　cleaning. To clean the seeds, we incubated seeds and locule contents at 24°C in 1%

754　protease solution (Rapidase C80 Max) for 2h, then rinsed them in deionized water and

755　air-dried. We then stored seeds in a cool, dry, dark location until use.

756　　　To grow plants for detached leaf assays, we bleach-sterilized all seeds and

757　germinated them on paper in the growth chamber using flats covered with humidity

758　domes. At 7 days we transferred seedlings to soil (SunGro Horticulture, Agawam, MA)

759　and grew all plants in growth chambers in 20°C, short-day (10h photoperiod) conditions

760　with 180-190 uM light intensity and 60% RH. We bottom-watered with deionized water

761　every two days for two weeks, and at week 3 watered every two days with added

762　nutrient solution (0.5% N-P-K fertilizer in a 2-1- 2 ratio; Grow More 4-18-38). The plants

763　were used for detached leaf assays 6 weeks after transferring seedlings to soil.

764　Flowering in this system did not occur until minimally 9 weeks of age for any accession,

765　and as such we were sampling midway between the juvenile/adult transition and any

766　flowering time decision. This window has been successful to minimize any major

767　ontogenetic effects on the pathogen/host interaction in other systems (Corwin,

768　Copeland et al. 2016).

769

770　***B. cinerea* genetic resources**

771　　　We utilized a previously described collection of *B. cinerea* isolates that were

772　isolated as single spores from natural infections of fruit and vegetable tissues collected

773　in California and internationally. (Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017). This

774　included five isolates obtained from natural infections of tomato. We maintained *B.*

775　*cinerea* isolates as conidial suspensions in 30% glycerol for long-term storage at -80°C.

776　For regrowth, we diluted spore solutions to 10% concentration in filter-sterilized 50%

777　grape juice, and then inoculated onto 39g/L potato dextrose agar (PDA) media. We

778　grew isolates at 25°C in 12h light and propagated every 2 weeks. Sequencing failed for

26

779 6 out of our 97 phenotyped isolates. For bigRR GWA mapping with the 91 isolates

780 genotyped in this study, we utilized a total of 272,672 SNPs against the *B. cinerea* T4

781 genome with minor allele frequency (MAF) 0.20 or greater, and less than 10% missing

782 calls across the isolates (SNP calls in at least 82/ 91 isolates). ~~For GEMMA mapping,~~

783 ~~we used 91 isolates with a total of 237,878 SNPs against the *B. cinerea* B05.10 genome~~

784 ~~with MAF 0.20 or greater and less than 10% missing calls. The overall SNP number~~

785 ~~was similar when using either reference genome.~~

786

787 **Detached leaf assay**

788 To study the effect of genetic variation in host and pathogen on lesion formation,

789 we infected detached leaves of 12 diverse tomato varieties with the above 97 *B. cinerea*

790 isolates. We used a randomized complete block design for a total of 6 replicates across

791 2 experiments. In each experiment, this included a total of 10 plants per genotype

792 randomized in 12 flats in 3 growth chambers. Each growth chamber block corresponded

793 with a replicate of the detached leaf assay, such that growth chamber and replicate

794 shared the same environmental block.~~ ~~At 6 weeks of age, we selected 5 leaves per

795 plant (expanded leaves from second true leaf or younger), and 2 leaflet pairs per leaf.

796 We randomized the order of leaves from each plant, and the leaflets were placed on 1%

797 phytoagar in planting flats, with humidity domes. Our inoculation protocol followed

798 previously described methods (Denby, Kumar et al. 2004, Kliebenstein, Rowe et al.

799 2005). Spores were collected from mature *B. cinerea* cultures grown on canned peach

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

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

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

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

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

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

806 room temperature. The entire inoculation took approximately 2 hour of time per

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

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

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

27

810

Automated Image Analysis

Lesion area was digitally measured using the EBImage and CRImage packages (Pau, Fuchs et al. 2010, Failmezger, Yuan et al. 2012) in the R statistical environment (R Development Core Team 2008), as previously described (Corwin, Copeland et al. 2016, Corwin, Subedy et al. 2016). Leaflets were identified as objects with green hue, and lesions were identified as low-saturation objects within leaves. Images masks were generated for both the leaf and lesion, then manually refined by a technician to ensure accurate object calling. The area of these leaves and lesions were then automatically measured as pixels per lesion and converted to area using a 1 cm reference within each image.

Data analysis

We analyzed lesion areas using ~~a~~ general linear model~~s~~ for the full experiment to determine the contributions of plant and pathogen genotype, ~~including the fixed effects of isolate genotype, plant domestication (*S. lycopersicum* or *S. pimpinellifolium*), plant genotype (which is nested within domestication status), experiment, and block (nested within experiment) on lesion area, as well as their interactions ((~~R lme4 package; (Bates, Maechler et al. 2015))~~.~~Two of our 97 isolates that did not have replication across 2 experiments were dropped at this stage of analysis. We used the following linear models throughout our analyses.

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

Where I represents fungal genotype (isolate), P represents plant genotype (accession), D represents domestication status, E represents experiment, W represents whole plant, L represents leaf, A represents leaflet position. Factors with the subscript R are included in the analysis as random effects.

842 ~~The significance of individual terms in the model did not change if experiment~~
843 ~~and block were treated as random effects. Adding terms for individual plant, leaf, and~~
844 ~~leaflet position did not significantly improve the full model, so they were omitted from~~
845 ~~further analysis.~~ ~~This~~ The within-plant accession model was used to calculate the
846 significance of each factor and to obtain the least-squared means of lesion size for each
847 *B. cinerea* isolate x tomato accession as well as for each *B. cinerea* isolate x
848 domestic<u>ated/</u> wild tomato. We also calculated a domestication sensitivity phenotype,
849 Sensitivity = (Domesticated lesion size – Wild lesion size) / Domesticated lesion size.
850 <u>We bootstrapped assignment of plant accessions to domestication groups in</u>
851 <u>order to assess the robustness of our observed domestication effects. We randomly</u>
852 <u>drew three genotypes from the domesticated and wild groupings and assigned them to</u>
853 <u>a new pseudo-wild grouping. The other six genotypes were assigned as a pseudo-</u>
854 <u>domesticated grouping and the model was rerun. This bootstrapping was repeated 100</u>
855 <u>times with each representing a random draw. We used these to repeat the full model</u>
856 <u>and to repeat the individual isolate models, as a test of the robustness of the tomato</u>
857 <u>domestication effect.</u>
858 Using tomato sequence data from the SolCAP diversity panel that contained 9 of
859 our 12 accessions, we determined pairwise genetic distances <u>between our accessions</u>
860 (Sim, Durstewitz et al. 2012). We calculated pairwise Euclidean distances between 426
861 wild and domesticated tomato accessions from Infinium SNP genotyping at 7,720 loci
862 using the R adegenet package (Jombart , Sim, Durstewitz et al. 2012). Clustering is by
863 R hclust (in the stats package) default UPGMA method (R Development Core Team
864 2008).
865 We used several methods to examine host specialization to tomato within *B.*
866 *cinerea*. First, we split our *B. cinerea* population into isolates collected from tomato
867 tissue vs. other hosts. We compared these groups by t-test for virulence on
868 domesticated tomato genotypes, wild tomato genotypes, or all tomato genotypes. Next,
869 we used a Wilcoxon signed-rank test to compare the rank order distribution of <u>model-</u>
870 <u>adjusted</u> lesion sizes across paired tomato genotypes. ~~To~~ <u>Also, to</u> examine host
871 specialization to tomato domestication within *B. cinerea*, we used a Wilcoxon signed-
872 rank test to compare the rank order of <u>model-adjusted</u> lesion sizes across all

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

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

875   significant response to plant genotype or domestication status.

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

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

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

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

880   2016, Francisco, Joseph et al. 2016, Kooke, Kruijer et al. 2016). The *B. cinerea* bigRR

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

882   described above. Because bigRR provides an estimated effect size, but not a p-value,

883   significance was estimated using 1000 permutations to determine effect significance at

884   95%, 99%, and (approximately) 99.9% thresholds (Doerge and Churchill 1996, Shen,

885   Alam et al. 2013, Corwin, Copeland et al. 2016). SNPs were annotated by custom R

886   scripts with gene transfer format file construction from the T4 gene models for genomic

887   DNA by linking the SNP to genes within a 2kbp window (http://www.broadinstitute.org,

888   (Staats and van Kan 2012)). Functional annotations are based on the T4 gene models

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

890   2012)). Additional genes of interest, based on a broad literature search of known

891   virulence loci, were taken from NCBI (https://www.ncbi.nlm.nih.gov/) and included by

892   mapping sequence to the T4 reference using MUMmer v3.0 (Kurtz, Phillippy et al.

893   2004).

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

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

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

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

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

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

900   ~~99%, and 99.9% thresholds (Doerge and Churchill 1996, Shen, Alam et al. 2013,~~

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

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

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

30

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

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

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

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

908 2012). Functional annotations of the overlap gene lists are based on the T4 gene

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

910 Kan 2012).

911

912 **Supplemental Data Files**

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

914 tomato accessions.

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

916 Results

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

918 Supplemental Data Set 4. Results of ANOVA following removal of domestication-

919 associated isolates

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

921 Supplemental Figure 21. Genetic distance between selected tomato accessions.

922 Supplemental Figure 3. Correlation between *B. cinerea* lesion size on tomato and on *A.*

923 *thaliana*

924 Supplemental Figure 42. Rank order plot of *B. cinerea* lesion size on two tomato

925 genotypes.

926

927 Supplemental Figure 3. GWA by GEMMA of *B. cinerea* lesion size on individual tomato

928 genotypes.

929 Supplemental Figure 4. Frequency of overlap in *B. cinerea* GEMMA GWA significance

930 across tomato accessions.

931 Supplemental Figure 5. GEMMA GWA analysis of domestication sensitivity in *B.*

932 *cinerea*.

933

934

Figure Legends

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

Figure 2. Distribution of tomato genotype susceptibility to infection with 97 genetically diverse *B. cinerea* isolates.
Violin plots show the distribution of lesion size caused by B. cinerea isolates on each tomato host genotype. Individual points are mean lesion size for each of the 97 different isolate-host pairs. The boxes show the 75th percentile distribution, and the horizontal line shows the mean resistance of the specific host genotype. The tomato genotypes are grouped based on their status as wild or domesticated germplasm.

Figure 3. Distribution of *B. cinerea* virulence by tomato domestication status.
The violin plots show the mean virulence of each *B. cinerea* isolate on the tomato genotypes, grouped as wild or domesticated germplasm. The domestication effect on lesion size is significant (Table 1 -ANOVA, p<2e-16=0.0006). The interaction plot between the two violin plots connects the average lesion size of a single *B. cinerea* isolate between the wild and domesticated germplasm.

Figure 4. GWA of *B. cinerea* lesion size on individual tomato genotypes.
Botrytis cinerea chromosomes are differentiated by shading, alternating light and dark grey.
a) Manhattan plot of estimated SNP effect sizes from bigRR for *B. cinerea* lesion size using a single tomato accession, LA2093. Permutation-derived thresholds are shown in horizontal dashed lines.
b) The number of tomato accessions for which a *B. cinerea* SNP was significantly linked to lesion development by bigRR using the 99% permutation threshold. Frequency is number of phenotypes in which the SNP exceeds the threshold. Vertical dotted lines

981 identify regions with overlap between the top 100 large-effect SNPs for LA2093 and
982 significance across the majority (≥6) of tomato genotypes tested.
983
984 **Figure 5. Frequency of overlap in *B. cinerea* GWA significance across tomato**
985 **accessions.**
986 a) The frequency with which the *B. cinerea* SNPs significantly associate with lesion size
987 on the 12 tomato accessions using bigRR and the 99% permutation threshold. The
988 black line indicates the expected frequency of random overlap, given the number of
989 significant SNPs per plant genotype and size of total SNP set. The inset zooms in on
990 the distribution for overlapping SNPs above 6 plant genotypes for easier visualization.
991 There were no SNPs expected to overlap by random chance in the inset.
992 b) The frequency with which *B. cinerea* genes significantly associated with lesion size
993 on the 12 tomato accessions. Genes were called as significant if there was one
994 significant SNP called at the 99% permutation threshold within the gene body, or within
995 2kb of the gene body.
996
997 **Figure 6. Host specificity of significant SNPs linked to the gene BcT4_6001**
998 **(Bcin14g00870).**
999 a) SNPs with effects estimates above the 99% permutation threshold are colored by
1000 trait (plant ~~phenotype~~ accession in which the effect was estimated). Wild accessions are
1001 oranges (yellow to red shades) and domesticated accessions are blues (green to purple
1002 shades). BcT4_6001 (Bcin14g00870) is a pectinesterase gene linked to at least one
1003 significant SNP on all 12 of the tested tomato accessions by bigRR. The annotated
1004 exons are depicted as turquoise rectangles, with the start codon marked with an arrow
1005 indicating the direction of transcription. Red rectangles indicate corresponding linkage
1006 disequilibrium blocks from Figure 6b.
1007 b) Linkage disequilibrium plot, including all pairwise comparisons of SNPs in the 2kb
1008 region surrounding Bcin14g00870. The color scheme for each SNP pair is D'/LOD:
1009 white if LOD <2 and D' <1, bright red for LOD ≥2 and D'=1, intermediate shades for
1010 LOD≥2 and D'<1.
1011
1012 **Figure 7. GWA analysis of domestication sensitivity in *B. cinerea*.**
1013 Domestication sensitivity of each isolate was estimated using the average virulence on
1014 the wild and domesticated tomato germplasm and using calculated Sensitivity. This was
1015 then utilized for GWA mapping by bigRR.
1016 a) The top 1000 SNPs that significantly affect lesion size across domesticated tomato,
1017 wild tomato or domestication sensitivity are shown. Significance is called as crossing
1018 the 99% permutation threshold.
1019 b) Venn diagram of overlapping SNPs identified as crossing the 99% permutation
1020 threshold for each trait.
1021 c) Venn diagram of overlapping genes identified as crossing the 99% permutation
1022 threshold for each trait. Genes were called as significant if there was one significant
1023 SNP within the gene body or within 2kb of the gene body.

1024

1024

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