**Crop domestication and pathogen virulence: The interaction of tomato domestication and *Botrytis cinerea* genetic diversity**

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**Abstract**

Human selection during crop domestication leads to shifts in numerous traits, including resistance to biotrophic pathogens. Studies of qualitative resistance to biotrophic pathogens typically show decreased resistance in domesticated crops in comparison to their wild relatives. However, less is known about how crop domestication affects quantitative interactions with generalist pathogens. To study how crop domestication impacts interactions with generalist pathogens and correspondingly what is affected in the pathogen, we developed a population of the generalist pathogen *Botrytis cinerea* and infected this population on wild and domesticated tomato accessions. We quantified variation in lesion size of 97 *B. cinerea* isolates on 6 domesticated *Solanum lycopersicum* and 6 wild *S. pimpinellifolium* genotypes. This showed that lesion size variation is significantly controlled by plant domestication status, plant genotype, and pathogen genotype. The effect of domestication status was slightly elevated resistance in the wild germplasm, but interestingly there was no evidence of a bottleneck in these accessions, with wild and domestic tomatoes showing a similar range of resistance. To complement this, we conducted genome-wide association (GWA) in *B. cinerea* that found a highly quantitative basis of virulence on tomato. This collection of genes was highly specific to distinct tomato accessions, suggesting that breeding against this pathogen would need to utilize a diversity of isolates to capture all possible mechanisms. There was a specific subset of *B. cinerea* genes that linked to altered virulence against the wild versus domesticated tomato accessions. This study begins to identify novel potential virulence mechanisms for this generalist pathogen, and generates hypotheses for the effect of plant domestication on B. cinerea virulence. Future studies may test whether these mechanisms and hypotheses hold for additional diverse hosts of *B. cinerea*.

**Introduction**

The progression of a plant disease is mediated by complex interactions among diverse host and pathogen molecular pathways. The resulting disease outcome is the sum of pathogen virulence/sensitivity and host plant susceptibility/resistance mechanisms, specific to the interacting genotypes of the host and pathogen. A key aspect controlling the genetic architecture of this interaction is 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 genes for plant resistance to specialist pathogens confer qualitative resistance through plant innate immunity, via large-effect loci that enable the recognition of the pathogen by the plant {Dodds 2010; Pieterse 2012; Dangl 2001; Jones 2006}. 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, but in turn are detected by plant proteins that guard the signaling networks {Ferrari 2007; Dodds 2010; Jones 2006; Boller 2009; Bittel 2007}. The evolution of these 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 diverse plant hosts. Generalist pathogens may have less stringent co-evolution in connection to specific hosts and their accompanying resistance mechanisms, because these pathogens can easily shift from host to host. Thus, generalist pathogens can evade the rapid evolution of new resistance mechanisms within specific hosts until they evolve to counter this new resistance. This niche-shifting ability may partly explain the observation that most natural resistance to generalist pathogens is highly polygenic and the underlying plant genes for resistance are quantitative {Rowe 2008; Corwin 2016; Glazebrook 2005; Goss 2006; Barrett 2009; Nomura 2005}. Unlike qualitative resistance loci that predominantly involve genes in signaling cascades, the quantitative resistance genes to generalist pathogens also include a broad array of direct defense genes like those involved in secondary metabolite production, cell wall formation, and defense proteins {Ferrari 2007; Poland 2009; Denby 2004; Zipfel 2004; Walz 2008; Zheng 2006; Rowe 2008; Corwin 2017}. Importantly, these quantitative plant resistance loci do not alter resistance to all isolates of a pathogen but are dependent upon the infecting pathogen’s genotype. For example, the ability of the *Arabidopsis* defense metabolite, camalexin, to provide resistance to *Botrytis cinerea* depends upon if the specific isolate is sensitive or resistant to camalexin {Kliebenstein 2005}. In contrast to the polygenic nature of plant resistance, little is known about the genetic architecture of virulence within generalist pathogens and how this is affected by genetic variation in the pathogen. There are no reported naturally variable large-effect virulence loci in generalist pathogens, suggesting that virulence is controlled by quantitative genetic variation in these pathogens. This potential for genetic co-dependency between generalist pathogen and host plant suggests that we need to work with genetic variation in both the host and pathogen to truly understand quantitative host-pathogen interactions.

A key evolutionary process in plants that has affected resistance to specialist pathogens is domestication from wild plants to crop plants. Domesticated plant varieties are typically more sensitive to specialist pathogens than are their wild relatives {Smale 1996; Rosenthal 1997; Couch 2005; Dwivedi 2008}, and pathogens may evolve higher virulence on domesticated hosts {Stuckenbrock 2008}. Further, domestication typically imposes a strong genetic bottleneck that reduces genetic diversity in the crop plant, and often decreases the availability of resistance alleles against specialist pathogens in the crop plant germplasm {Tanksley 1997; Doebley 2006; Chaudhary 2013}. These general evolutionary patterns, of lower resistance and allelic diversity found when studying the interaction of specialist pathogens with crop plants, are assumed to similarly hold for generalist pathogens and their domesticated hosts. However, we have 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 conduct a detailed analysis of how domestication may alter the interaction of a plant with a broad generalist pathogen and correspondingly 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 pre- and post-harvest crop losses in many plant species {Nicot 1996; Elad 2007; Fillinger 2015}. Individual isolates of *B. cinerea* display the same broad host range as the generalist species {Deighton 2001; Finkers 2007; Ten Have 2007; Corwin 2016} in contrast to pathogens like *Fusarium oxysporum* where the species can infect diverse hosts, but each isolate is highly host specific {Katan 1999; Barrett 2012}. Additionally, *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 2005; Dalmais 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 {Schumacher 2012; Rowe 2007}. This genetic variation in diverse virulence mechanisms can contribute to the formation of quantitative differences in virulence between the isolates {ten Have 1998}. In support of this is genomic sequencing of diverse *B. cinerea* isolates that found a high level of genomic sequence diversity spread across the genome. The polymorphism rate in *B. cinerea* is 6.6 SNP/kb in this study which is more variable than previously studied plant pathogens (1-2 SNP/kb in *Blumeria graminis*, 5.5 SNP/kb in the compact genome of the obligate biotroph Plasmodiophora brassicae) {Hacquard 2013; Wicker 2013}, and on par with the genetic diversity found in the human pathogen *Mycobacterium tuberculosis* (2.9 to 6.2 SNP/kb){Power 2017; Farhat 2013; Desjardins 2016}. The genomic sequencing of these isolates showed that the species has a high level of recombination and genomic admixture. 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. This can potentially identify the pathogen variation controlling quantitative virulence even in non-model plant systems.

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 2012; Hahn 2014; Romanazzi 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 {Finkers 2007; Rowe 2008; Corwin 2016}. Tomato is a model system for study of the impact of domestication upon plant physiology and resistance {Panthee 2010; Bergougnoux 2014; Tanksley 2004; Bai 2007}. This includes evidence that tomato domestication has altered the circadian clock phase {Muller 2016}, which can modulate resistance to *B. cinerea* {Sauerbrunn 2004; Weyman 2006; Bhardwaj 2011; Hevia 2015}. This suggests that host plant domestication within tomato can alter traits known to influence *B. cinerea* resistance from other systems. Thus we are using the tomato-*B. cinerea* pathosystem to directly measure the interaction of crop domestication with genetic variation in a generalist pathogen to better understand the evolution of this pathosystem.

In this study, we infected 97 genetically diverse *B. cinerea* isolates on a collection of domesticated tomato, *S. lycopersicum*, and wild tomato, *S. pimpinellifolium*, and measured lesion size. We examined the contributions of tomato variation, domestication, and *B. cinerea* genetic variation to lesion size in on detached leaves. Lesion size of *B. cinerea* is a quantitative trait that was controlled by plant domestication status, plant genotype and pathogen genotype. We did not find evidence for host specialization; *B. cinerea* isolates collected from tomato tissues are not within the most-virulent isolates on tomato. Our findings indicate that while all isolates are generalists across domestication in *Solanum,* a subset of single isolates are sensitive to tomato domestication. We then conducted genome-wide association (GWA) in *B. cinerea* to identify the pathogen loci where genetic variation is sensitive to host phenotypic variation, and more specifically to domestication. At the genetic level, virulence of *B. cinerea* is highly quantitative, with hundreds of significant SNPs with small effect sizes associated with lesion area on each tomato genotype. Importantly, there is a subset of loci in the pathogen that are critically sensitive to domestication in the crop, and could be tools for improved breeding as well as to interrogate how domestication in tomato has influenced generalist pathogen resistance.

**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*) from throughout its native range (Peru, Ecuador) and 6 heritage and modern varieties of *S. lycopersicum*. 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.

***B. cinerea* genetic resources**

We utilized a previously described collection of *B. cinerea* isolates that were collected as single spores collections from natural infections of fruit and vegetable tissues collected in California and internationally {Atwell 2015; Zhang 2017}. 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 GWA mapping with the 91 isolates genotyped in this study, we utilized a total of 272,672 SNPs with MAF 0.20 or greater, and less than 10% missing calls across the isolates (SNP calls in at least 82/ 91 isolates).

**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 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 older), 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 2004; Kliebenstein 2005}. Spores were collected from mature *B. cinerea* cultures grown on canned peach plates, and diluted to 10 spores/ µL in filter-sterilized 50% organic grape juice. 4µl droplets of the diluted spore suspensions were placed onto the detached leaflets at room temperature. Mock-inoculated control leaves were treated with 4µL of 50% organic grape juice without spores. Digital photos were taken of all leaflets at 24, 48, and 72 hours post inoculation and automated image analysis was used to measure lesion size.

**Automated Image Analysis**

Lesion area was digitally measured using the EBImage and CRImage packages {Pau 2010; Failmezger 2010} in the R statistical environment {R Development Core Team and Team 2009}, as previously described {Corwin 2016 PLOS; Corwin 2016 APS}. 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 for the full experiment, 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 (lme4; {Bates 2015}). Two of our 97 isolates did not have replication across 2 experiments, so they were dropped at this stage of analysis. There was no difference in the results if experiment and block were treated as random effects. Adding terms for individual plant, leaf, and leaflet position did not significantly improve the full model, so they were omitted them from further analysis. We also tested a mixed model with random effects of experiment and block, but this did not affect our interpretation of the fixed effects. This model was used to calculate the significance of each factor and to obtain the least-squared means of lesion size for each *B. cinerea* isolate x tomato accession as well as for each *B. cinerea* isolate x domestic/wild tomato.

These means were used as the phenotypic input for GWA using bigRR, a heteroskedastic ridge regression method that incorporates SNP-specific shrinkage {Shen 2013}. This approach has previously had a high validation rate {Ober 2015; Corwin 2016; Francisco 2016; Kooke 2016}. The *B. cinerea* GWA used 272,672 SNPs at MAF 0.20 or greater and <10% missing SNP calls as described above. Because bigRR provides an estimated effect size, but not a p-value, significance was estimated using 1000 permutations to determine effect significance at 95%, 99%, and 99.9% thresholds {Doerge 1996; Shen 2013; Corwin 2016}. SNPs were annotated using SNPdat {Doran 2013} with gene transfer format file construction from the T4 gene models for genomic DNA by linking the SNP to genes within a 2kbp window ([http://www.broadinstitute.org](http://www.broadinstitute.org/), {Staats 2012}). Functional annotations are based on the T4 gene models for genomic DNA (http://www.broadinstitute.org, *B. cinerea*; {Staats 2012}). Additional genes of interest were taken from NCBI (https://www.ncbi.nlm.nih.gov/) and included by mapping sequence to the T4 reference using MUMmer v3.0 {Kurtz 2004}. We used the program InterProScan within BLAST2GO for functional gene ontology (GO) annotation of the gene models (http://www.blast2go.com).

**Results**

**Experimental Design**

To directly quantify how tomato domestication affects quantitative resistance to a population of a generalist pathogen, we infected a collection of 97 diverse *B. cinerea* isolates (genotypes) on 6 wild and 6 domesticated tomato genotypes. Previous studies have examined *B. cinerea* resistance between domesticated and distantly related wild tomato species (i.e. *S. lycopersicum* and *S. pimpinellifolium*) using single isolates of pathogens {Egashira 2000; Nicot 2002; Guimaraes 2004; Ten Have 2007; Finkers 2008}. These previous studies typically used individual wild and domesticated tomato accessions that were the founders of mapping populations, and found a wide range of *B. cinerea* resistance levels. However, it is still unknown how domesticated and closely related wild tomatoes compare for *B. cinerea* resistance using multiple plant genotypes and a population of the pathogen. We selected 6 domesticated *Solanum lycopersicum* and 6 wild *S. pimpinellifolium* accessions, the closest wild relative of *S. lycopersicum*, to directly study how domestication has influenced resistance to *B. cinerea* {Peralta 2008}. For the pathogen population, we used a previously collected sample of 97 *B. cinerea* isolates obtained from various eudicot plant hosts, including tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2, Supersteak). 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. We digitally measured the area of all developing lesions at 72 hours post infection (HPI) (Figure R1). At 72 hours significant lesion growth was visible, but no lesions had spread to infect over half of the leaflet. 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 2003; Denby 2004; Kliebenstein 2005; Ferrari 2007; Rowe 2008}.

**Comparison of Pathogen Genetics, Plant Genetics and Crop Domestication Effects on Resistance**

To measure the relative contribution of genetic diversity in both the plant and the pathogen to variation in the virulence/ resistance phenotype, we used a multiple linear regression model {R Core 2013}. This model directly tested the contribution of plant genotype, plant domestication status, and pathogen genotype (isolate) to variation in lesion size. The final model explained 60% of the variance for lesion size, and showed that genetic variation within both the host plant and the pathogen had significant effects on lesion growth, but pathogen isolate diversity explained 3.5x more of the variance than plant genotype, 10.2% of total variance for pathogen isolate vs. 2.9% for plant genotype (Table R1 and Figure R4A). Interestingly, tomato domestication status significantly impacted *B. cinerea* virulence, as shown by the small but significant effects of genetic variation between domesticated and wild tomatoes (<1% of total variance, Table R1). There was no evidence for significant interaction effects between pathogen isolate and plant genotype, but this term contributed the largest proportion of the plant-related variance in lesion size (7.5% of total variance, Table R1). This lack of significance may be due to the vast number of degrees of freedom in this term (Table R1). Thus, the interaction between tomato and *B. cinerea* was significantly controlled by genetic diversity within the host plant and the pathogen, including a slight effect of domestication status.

**Domestication and Lesion Size Variation**

Existing literature predominantly reports that crop domestication decreases plant resistance to pathogens {Stukenbrock 2008; Smale 1996; Couch 2005; Rosenthal 1997; Dwivedi 2008}. In our analysis, we identified a significant difference in the resistance of wild and domesticated tomato to the population of *B. cinerea* isolates (p <2e-16, Table R1). This agrees with the hypothesis that domestication decreases resistance, as the average lesion size was slightly greater (18% increase) on domesticated than on wild tomato genotypes (Table R1, Figure R2). 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 (2.9% vs. 0.8% of total variance, Table R1). So while we did observe the expected increase of susceptibility in domesticated tomato, domestication did not predominantly explain the effects of tomato genotype on lesion size variation and there was significant genetic variation within both wild and domestic tomato species for *B. cinerea* resistance.

In addition to altering trait means, domestication commonly decreases genetic variation in comparison to wild germplasm due to bottlenecks during domestication, including for tomato {Tanksley 1997; Doebley 2006; Bai 2007}. This decreased genetic variation should also limit phenotypic variation, including disease phenotypes. Interestingly in this tomato population, the domesticated tomato genotypes had a wider range of average lesion size than wild genotypes; the 90th percentile range (95th percentile to 5th percentile) was 2.03 cm2 lesion size variation on domesticated tomato versus 1.76 cm2 variation on wild tomato. Additionally, the wild and domesticated tomato genotypes showed statistically similar variation in resistance (F-test, F=1.39, 96 num df, 96 denom df, p=0.11)(Figure R3). Overall, there is a slight domestication impact on average resistance to *B. cinerea* that depended on the host genotype, but no evidence of a phenotypic bottleneck due to domestication.

**Pathogen Specialization to Source Host**

One evolutionary model of generalist pathogens suggests that isolates within generalist pathogen species may specialize on specific hosts. Alternatively, isolates may also be generalists, with specialization absent or occurring only at the gene level. Our collection of *B. cinerea* includes five isolates which 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 across all hosts 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, 9.7 df, p=0.14) (Figure R4E). In fact, one isolate collected from tomato tissue (KGB1) was within the 10 least-virulent isolates in this study (Figure R4E), and one was within the 10 most-virulent isolates (Triple3). This demonstrated significant genetic variation in virulence across the *B. cinerea* isolates, and that this collection of *B. cinerea* isolates are not strongly host-specific for tomato {Rowe 2007; Ma 2005; Stylianos 2012; Martinez 2003}.

**Pathogen Specialization to Host Variation**

Though we did not find evidence for *B. cinerea* adaptation to tomato based on isolate host source, the *B. cinerea* isolates may contain genetic variation at individual loci that allow them to better attack subsets of the tomato genotypes. A visual analysis of the data suggested an interaction between the genomes of *B. cinerea* and tomato (Figure R4). However, when using the full model, we found no significant interaction between isolate and individual host genotype, even though there was a large fraction of variance within each term (Table R1). This may indicate a lack of interaction between genetic variation in the host and pathogen. However, this negative result may also be because F-tests in factors with high degrees of freedom can be underpowered, as in the case of the isolate x plant genotype interaction term with 940 degrees of freedom (Table R1). To assess these two possibilities, we used an additional statistical approach to test for an interaction between *B. cinerea* and host genotype. We used a Wilcoxon signed-rank test to test if the rank of *B. cinerea* isolate-induced lesion size changed between pairs of tomato genotypes. This showed that when using the full isolate population, performance does significantly vary between host genotypes. When comparing mean lesion size between paired plant genotypes, 58% of tomato accession pairs significantly affected the distribution of lesion sizes across all isolates (Wilcoxon signed-rank test, Table R2, Figure R5). This pattern was consistent, irrelevant of whether we compared only domesticated host pairs, wild host pairs, or pairs across species (Wilcoxon signed-rank test, Table R2). As such, this suggests that the population of *B. cinerea* does display differential responses to the tomato genetic variation.

To test if specific *B. cinerea* isolates may be sensitive to domestication, we utilized an individual isolate ANOVA approach. The single-isolate ANOVAs including the fixed effects of plant, domestication, and experiment found two isolates with a significant effect of domestication on lesion size (p < 0.05, FDR corrected) (Figure R4F). These included one of the highly virulent isolates (Fd2), and one of the largely saprophytic isolates (Rose), suggesting that *B. cinerea* adaptation to tomato domestication is not dependent on isolate virulence. Both of these isolates were more virulent on domesticated than on wild tomato. Further, the Wilcoxon signed-rank test to compare the rank of mean lesion size of all the *B. cinerea* isolates on wild versus domestic tomato was significant (Wilcoxon signed-rank test, W = 5946, p-value = 0.002) (Figure R3). This suggests that in addition to the two highly domestication sensitive isolates that there is a broader pattern of *B. cinerea* specialization to tomato domestication.

**Quantitative Genetics of Pathogen Virulence on Tomato**

Genetic variation within *B. cinerea* had a large effect on virulence on tomato and responded to domestication within tomato. This suggests that there is genetic variation within the pathogen in which some alleles enhance and other alleles decrease virulence. To identify variable pathogen genes controlling differential virulence, we conducted a GWA mapping analysis within the pathogen. Due to the large effect of plant genotype on resistance to *B. cinerea*, we performed GWA using the model-corrected least-squared mean virulence measured on each tomato genotype as separate traits. We used a ridge-regression approach in combination with 272,672 SNPs from *B. cinerea* to estimate the phenotypic effects across the genome {Shen 2013; Corwin 2016 PLOS; Corwin 2016}. To determine significance of SNP effects, we permuted phenotypes 1000 times to calculate 95, 99, and 99.9% effect size thresholds within each plant host.

This GWA analysis showed that the genetic basis of *B. cinerea* virulence on tomato is highly polygenic. We identified from 1,284 to 25,421 SNPs within *B. cinerea* that were significantly associated with altered virulence on the 12 different host genotypes (SNP effect size estimate exceeded the 99% threshold). Interestingly, some of these *B. cinerea* SNPs were found for virulence on all of the different tomato genotypes, with 5 *B. cinerea* SNPs significantly linked to altered lesion size on all 12 tomato accessions (Figure R6B). This is much higher than the expected overlap of our SNP sets across tomato accessions due to random chance (Figure R7A). 215 SNPs were called in at least 10 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. Changing from a SNP-by-SNP focus to looking at *B. cinerea* genes, using a 2kbp window within each of SNPs associated with more than 6 of the 12 phenotypes, found 18 genes linked to differential virulence in all 12 tomato accessions. A further 377 genes were linked to differential virulence on 7 to 11 tomato accessions. This is indicative of multiple haplotypes contributing to virulence at the candidate genes, with individual SNPs sampling unique haplotypes within a region (Figure R8). Significant SNPs at a single cerato-platanin gene (BcT4\_4591) vary in direction of effect depending on tomato host genotype, suggesting at least 3 haplotypes contributing to lesion size in this region. These findings suggest that there is significant genetic variation in *B. cinerea* virulence that is dependent upon the host’s genetic background, which is in agreement with the fraction of variation attributed to this term in the linear model. Thus, the pathogen appears to rely on polygenic small effect loci to customize virulence on the different tomato hosts.

Of the 18 genes with SNPs significantly associated with *B. cinerea* virulence on all 12 tomato genotypes, four are enzymes, one is involved in signal transduction (BcT4\_10373, Bcin08g01740), and one is a cerato-platanin (BcT4\_4591) (Table S1). There are eight functional annotations significantly overrepresented among genes associated with the 12 plant traits, including five enzymes, signal transduction, and cerato-platanin (Table S1).

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

To directly map *B. cinerea* genes that control differential virulence on wild and domestic tomatoes, we used the least-squared mean virulence of each isolate on 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 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 domestic tomato hosts identified a complex pattern of significant SNPs similar to the individual tomato accessions (Figure R6). This had a high degree of overlap between the two traits. In contrast, the Domestication Sensitivity trait identified a much more limited set of SNPs that had less overlap with either the mean lesion area on Domesticated or Wild tomato (Figure R6). To begin querying the underlying gene functions for these various *B. cinerea* loci, we called genes as significant if there was a SNP within 2kb of that gene (Figure R9C). Using all 1935 genes linked to domestication in a GO enrichment analysis found only 17 biological functions as significantly overrepresented (Fisher exact test, p=0.05; Table S1) when compared to the whole-genome annotation of 14539 genes. Nine functional annotations are overrepresented for sensitivity genes, and six of these are involved in metabolism (Table S1). The additional eight functions overrepresented for domestication traits include enzymes, signaling, and mRNA splicing. Metal ion binding, transport, catalysis, and gene silencing are uniquely overrepresented in *B. cinerea* growth on wild tomato genotypes. None of the overrepresented functions include classical virulence or pathogenicity annotations. Thus, the genetic architecture of how *B. cinerea* responds to tomato domestication appears to be polygenic, with many loci of trait-dependent small effect sizes. But, there is an apparent subset of *B. cinerea* genes that may be specific to the genetic changes that occurred in tomato during domestication.

**DISCUSSION**

The genetics of plant resistance to generalist pathogens are mostly quantitative, depend upon pathogen genotype, and rely on genetic variation in both signal perception and direct defense genes {Rowe 2008; Corwin 2016}. Previous studies on tomato resistance to *B. cinerea* have found a quantitative genetic architecture that varies between domesticated and wild tomato species {Egashira 2000; Nicot 2002; Guimaraes 2004; Finkers 2007; Ten Have 2007; Finkers 2008}. However, it was unclear how this pattern is reiterated when using the most closely related wild tomato, *S. pimpinellifolium*. Further, it was not known how the choice of *B. cinerea* isolate may change this interaction. In this study, we used genetic variation in wild and domesticated tomato accessions in conjunction with a population of *B. cinerea* isolates to test these questions, and further to test how domestication within tomato may have 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, is significantly affected by pathogen genotype, host genotype, and domestication status (Table R1). Tomato domestication led to a slight but significant decrease in resistance to the pathogen but critically, there was no evidence of a domestication bottleneck, with the wild and domestic tomato accessions having similar variance in resistance (Table R1, Figure R2). 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 R4). The genetics underlying *B. cinerea* virulence on tomato are highly quantitative, and vary with tomato genotype and domestication status (Figure R7, Figure R9). Some genes contribute to virulence on most of the hosts tested, and we find some evidence for domestication-sensitive genes within *B. cinerea*.

**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 contributes less than 1% of the total variance of *B. cinerea* lesion size on tomato (Table R1). However, domestication status alone was 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. The effect of tomato domestication varied across the *B. cinerea* isolates, with specific isolates and loci linked to differential virulence across wild and domestic tomatoes. This supports the approach of studying natural variation within *B. cinerea* to understand the factors contributing to *B. cinerea* virulence and how this is altered by crop domestication. Studies of few isolates could miss the host domestication effect entirely, or provide a false positive signature of uniformly elevated virulence on domesticated hosts.

Host domestication is theoretically expected to decrease resistance to pathogens as alleles are lost in the domestication bottleneck as found for specialist pathogens [GIVE EXAMPLES]. Surprisingly, we did not find evidence for a domestication bottleneck in the phenotypic resistance to *B. cinerea*. This is in contrast to previous studies that explicitly show that there is a genotypic bottleneck within tomato domestication {Miller 1990; Koenig 2013}. This suggests that at least for this generalist pathogen, the genetic bottleneck of tomato domestication has not imparted a phenotypic bottleneck. One possible explanation is that resistance to this pathogen is so polygenic in the plant that our experiment is not sufficiently large to pick up any genetic bottleneck effect using phenotypic variance. These patterns, of mild increase in resistance to *B. cinerea* due to plant domestication, and within-species plant variation exceeding the contribution of domestication itself, may be unique to interactions between *B. cinerea* and tomato, or more general. It remains to be seen if these patterns hold for *B. cinerea* on its other host plants. It is unclear whether domestication has a universal effect on plant resistance to *B. cinerea*, or if each domestication event is unique.

**Polygenic quantitative virulence and breeding complications**

Our results indicate a highly polygenic basis of quantitative virulence of the generalist *B. cinerea* on tomato. The variation in lesion size is linked to numerous *B. cinerea* SNPs, each with small effect sizes (Figure R6a). Importantly, the tomato host accession greatly influenced which loci were significantly associated to lesion size in *B. cinerea* (Figure R7). Thus, it possible that different alleles within the pathogen link to differential virulence on specific host genotypes. This polygenic architecture of virulence is distinctly different from specialist pathogens that often have one or a few large effect genes that control virulence {De Feyter 1992; Keen 1992; Abramovitch 2004; Vleeshouwers 2014; Boyd 2013} but see {Lannou 2012}. It is possible that the SNP effect estimates are deflated, and number of contributing SNPs inflated, if individual SNPs are sampling several different haplotypes in the regions associated with *B. cinerea* lesion size. Further studies are needed to test the relationships between SNP and haplotype effect size estimates in *B. cinerea* and to compare how the host plant species may affect this image of genetic variation in virulence.

Our results indicate some particular challenges for breeding durable resistance to *B. cinerea* and possibly other generalist pathogens. In combination with genomic sequencing showing that this pathogen is an inter-breeding population, this suggests that the pathogen is blending a large collection of polymorphic virulence loci. Thus, it is not sufficient to breed crop resistance against a single isolate of *B. cinerea*, as this resistance mechanism would likely be rapidly overcome by new genotypes within the field population of *B. cinerea*. In contrast, it is likely necessary to breed resistance using a population of the pathogen, and to focus on plant loci that target entire pathways or mechanisms. The results in this study indicate that the specific genetics of the plant host, the general domestication 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. As such, utilizing a single or even a few pathogen genotypes to guide resistance breeding in plants is unlikely to translate to durable resistance against *B. cinerea* as a species. However, the lack of a domestication bottleneck on tomato resistance to B*. cinerea* suggests that, at least for tomato, the domesticated germplasm has sufficient resistance alleles and it is not necessary to introgress genes or alleles from wild relatives to improve resistance.

**Molecular mechanisms and polygenic virulence**

GWA mapping of genes and SNPs controlling differential virulence in *B. cinerea* began to identify new mechanisms and loci that may play key roles in controlling differential virulence in this generalist pathogen. The mechanisms of quantitative virulence identified in this study are in contrast to previously-described qualitative virulence loci. The GO enrichments that we identified using the GWA were enzymatic pathways, protein degradation and transport processes (Table S1). Through analysis of the genes significantly associated with all 12 plant traits, we identified a single cerato-platanin gene (BcT4\_4591; Figure R8), a potential PAMP {Baccelli 2014; Gaderer 2014; Pazzagli 2014}. Fungal cerato-platanins have been linked to induction of systemic acquired resistance and defense compound biosynthesis in plants {Frias 2012; Scala 2004}. Interestingly, using specific *a priori* gene searches, we did not identify any other known fungal MAMPs or PAMPs, i.e. chitin, mannans, glycans or glycolipid genes, as loci contributing to variation in virulence across tomato accessions (CITATIONS){Corwin 2016; Corwin 2017}. We also did not identify known virulence loci such as NEPs, VELVET or polygalacturonases (CITATIONS). All of these genes did have SNPs within the analysis, but it is possible that the size of the population was simply not powerful enough to identify these loci. Thus, this GWA mapping in the pathogen is allowing the identification of new potential virulence mechanisms. Several of the functions we identified are suggestive of pathogen virulence. Through analysis of loci contributing to virulence on all 12 host genotypes, we identified a terpene synthase (Table S1). Reduced terpene biosynthesis has been linked to viral infections and susceptibility to whiteflies in plants {Li 2014}. Through analysis of domestication-sensitive loci, we identified genes that may control production, transport or perception of kyneurine (Table S1). Kyneurine induces apoptosis through reactive oxygen species mediated pathways in mammalian cells {Song 2011}, and *B. cinerea* kyneurine biosynthesis could similarly be involved in plant cell death, via a pathway that was altered over the course of tomato domestication. We also identified betalain biosynthesis as an overrepresented function among domestication-sensitivity loci (Table S1). Betalain production in plants enhances resistance to B. cinerea {Polturak 2017}, so B. cinerea may be interacting with this signaling pathway in a domestication-dependent manner.

**Conclusion**

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 what happens 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 if independent crop domestication events have a consistent underlying genetic signal of *B. cinerea* adaptation to plant domestication.

**Tables**

**Table R1. ANOVA results of the interaction between 12 tomato accessions and 95 *B. cinerea* isolates measured as lesion area.**

The Type III Sums-of-Squares, F-value, Degrees of Freedom and p-value for the linear modelling of lesion area for 12 tomato accessions by 95 *B. cinerea* isolates is shown. Two of our 97 isolates did not have replication across 2 experiments, so they were dropped at this stage of analysis. The terms are as follows; Isolate is the 95 *B. cinerea* isolates, Domestication is wild tomato, *S. pimpinellifolium*, versus domestic tomato, *S. lycopersicum*, Plant is 12 tomato genotypes nested within their respective domestication groupings, Experiment tests the 2 independent replicate experiments, Experiment/Block tests the three blocks nested within each experiment. In addition interactions of these factors are tested (:).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Fixed Effect | SS | F value | DF | p |
| Isolate | 256.6 | 13.54 | 94 | **<2e-16** |
| Domestication | 19.45 | 96.46 | 1 | **<2e-16** |
| Domest/Plant | 73.67 | 36.54 | 10 | **<2e-16** |
| Iso:Domest | 20.67 | 1.091 | 94 | 0.260 |
| Iso:Domest/Plant | 189.5 | 0.9838 | 940 | 0.623 |
| Experiment | 545.7 | 2707 | 1 | **<2e-16** |
| Exp/Block | 201.0 | 249.3 | 4 | **<2e-16** |
| Exp:Iso | 152.2 | 8.028 | 94 | **<2e-16** |
| Exp:Domest | 0.83 | 4.095 | 1 | 0.043 |
| Exp:Domest/Plant | 47.43 | 23.53 | 10 | **<2e-16** |

**Table R2. Correlation of lesion area caused by the 97 *B. cinerea* isolates across all of the tomato accessions.**

FDR corrected p-values of Wilcoxon signed-rank test comparing mean *B. cinerea* lesion area on pairs of tomato accessions. Bold text indicates significance at p<0.01 after correction, italicized text indicates suggestive p-values p<0.1 >0.01. NS shows non-significant interactions.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Wild |  |  |  |  |  | Domest |  |  |  |  |  |
|  |  | LA1547 | LA1589 | LA1684 | LA2093 | LA2176 | LA480 | LA2706 | LA3008 | LA3475 | LA410 | LA4345 | LA4355 |
| Wild | LA1547 |  | 3256 | 3975 | 3069 | 2228 | 3006 | 2165 | 3478 | 1847 | 1253 | 3942 | 2970 |
|  | LA1589 | **<0.001** |  | 5323 | 4755 | 3566 | 4563 | 3396 | 4860 | 3173 | 2406 | 5491 | 4426 |
|  | LA1684 | *0.086* | NS |  | 4020 | 3008 | 3926 | 2885 | 4194 | 2627 | 1959 | 4776 | 3810 |
|  | LA2093 | **<0.001** | NS | NS |  | 3395 | 4575 | 3260 | 4943 | 2919 | 2093 | 5630 | 4384 |
|  | LA2176 | **<0.001** | **0.007** | **<0.001** | **0.002** |  | 5766 | 4428 | 5955 | 4287 | 3411 | 6670 | 5610 |
|  | LA480 | **<0.001** | NS | *0.067* | NS | **0.012** |  | 5939 | 4376 | 6212 | 6999 | 3703 | 4869 |
| Domest | LA2706 | **<0.001** | **0.002** | **<0.001** | **<0.001** | NS | **0.003** |  | 6071 | 4564 | 3785 | 6716 | 5794 |
|  | LA3008 | **0.003** | NS | NS | NS | **0.003** | NS | **0.001** |  | 3062 | 2339 | 5309 | 4283 |
|  | LA3475 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | NS | **<0.001** | NS | **<0.001** |  | 3824 | 7088 | 6022 |
|  | LA410 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.002** | **<0.001** | *0.030* | **<0.001** | *0.037* |  | 7779 | 6820 |
|  | LA4345 | *0.072* | *0.065* | NS | *0.030* | **<0.001** | *0.018* | **<0.001** | NS | **<0.001** | **<0.001** |  | 3601 |
|  | LA4355 | **<0.001** | NS | *0.034* | NS | *0.032* | NS | **0.010** | NS | **0.002** | **<0.001** | **0.009** |  |

**Figures**

**Figure R1. *Botrytis cinerea* x tomato detached leaf assay and digital image analysis.** 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.

A) 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.

**Figure R2. Distribution of tomato genotype susceptibility toinfection 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 R3. 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 R1 ANOVA, p<2e-16). 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 R4. Highlighted variance of diversity in *B. cinerea* x tomato interctions.**

Shown is an interaction plot of lesion size due to individual *B. cinerea* isolates on all of the tomato host genotypes, grouped by domestication status. The x-axis includes each tomato host genotype. Each line traces the average lesion size of a single *B. cinerea* isolate across hosts.

A) Plot of all isolates.

B) The common reference *B. cinerea* isolate B05.10 is highlighted in black.

C) The ten highest-virulence isolates, as estimated by mean virulence across all tomato genotypes, are highlighted in black.

D) The ten most saprophytic, or low virulence, isolates, as estimated by mean virulence across all genotypes, are highlighted in black.

E) The five isolates collected from tomato tissue are highlighted in black.

F) The two isolates with significant domestication sensitivity are shown in black.

**Figure R5. Rank order plot of B. cinerea lesion size on two tomato genotypes.**

Each B. cinerea isolate is a straight line tracing mean lesion size on LA1547 to mean on LA0410, the two host genotypes with the most pronounced effect on the lesion size distribution across all isolates (Wilcoxon signed-rank test, p < 7.18e-17, Table R2). Isolate rank order shifts from

LA1547 to LA0410, as most isolates are more virulent on LA0410 but a significant subset reverse this trend. A total of 38 of the 66 plant host pairs display this pattern of shifting isolate rank order.

**Figure R6. GWA of *B. cinerea* lesion size on individual tomato genotypes.**

*Botrytis cinerea* chromosomes are differentiated by shading, alternating black and grey.

A) Manhattan plot of estimated SNP effect sizes 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 using the 99% permutation threshold. Frequency is number of phenotypes in which the SNP exceeds the threshold. Vertical dotted lines identify regions with overlap between the top 100 large-effect SNPs for LA2093 and significance across the majority (≥6) of tomato genotypes tested.

**Figure R7. Frequency of overlap in *B. cinerea* GWA significance across tomato accessions.**

A) Frequency with which the *B. cinerea* SNPs significantly associated with lesion size on the 12 tomato accessions using the 99% permutation threshold. Black lines indicate the expected frequency of overlap, given the number of significant SNPs per plant genotype and size of total SNP set.

B) Frequency with which a *B. cinerea* gene significantly associated with lesion size on the 12 tomato accessions. Genes were called as significant if there was one significant SNP in the top 1000 called at the 99% permutation threshold within the gene body, or within 2kb of the gene body.

**Figure R8. Host specificity of significant SNPs linked to the gene BcT4\_4591.**

SNPs with effects estimates above the 99% permutation threshold are colored by trait (plant phenotype in which the effect was estimated). BcT4\_4591 is a cerato-platanin gene linked to at least one significant SNP on all 12 of the tested tomato accessions. The annotated exons are depicted as turquoise rectangles.

**Figure R9. GWA analysis of domestication sensitivity in *B. cinerea*.**

Domestication sensitivity of each isolate was estimated using the average virulence on the wild and domesticated tomato germplasm using Sensitivity = (Domesticated lesion size – Wild lesion size) / Domesticated lesion size. This was then utilized for GWA mapping.

A) The top 1000 SNPs that significantly affect lesion size across domesticated tomato, wild tomato or domestication sensitivity are shown. Significance is called as crossing the 99% permutation threshold.

B) Venn diagram of overlapping SNPs identified as crossing the 99% permutation threshold for each trait.

C) Venn diagram of overlapping genes identified as crossing the 99% permutation threshold for each trait. Genes were called as significant if there was one significant SNP within the gene body or within 2kb of the gene body.

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