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

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**Keywords: Botrytis cinerea, plant-pathogen interaction, tomato, domestication; generalist pathogen; genome wide association mapping**

**Abstract**

Human selection during crop domestication alters numerous traits, including disease resistance. Studies of qualitative resistance to specialist pathogens typically find 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 plant resistance to generalist pathogens, and correspondingly how this interacts with the pathogen’s genetics, we infected a collection of wild and domesticated tomato accessions with a genetically diverse population of the generalist pathogen *Botrytis cinerea*. We quantified variation in lesion size of 97 *B. cinerea* genotypes (isolates) on 6 domesticated *Solanum lycopersicum* and 6 wild *S. pimpinellifolium* genotypes. This showed that lesion size is significantly controlled by plant domestication, plant genetic variation within the domestication groups, and the pathogen’s genotype. Overall, resistance was slightly elevated in the wild germplasm in comparison to domesticated tomato accessions, but interestingly there was no evidence of decreased variation in resistance to *B. cinerea* associated with a domestication-associated genetic bottleneck in tomato. Genome-wide association (GWA) mapping in *B. cinerea* identified a highly polygenic collection of genes where alleles modulated virulence on distinct tomato accessions. This suggests that breeding against this pathogen would need to utilize a diversity of 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. This indicates that this generalist pathogen already has the necessary allelic variation in place to handle the introgression of wild resistance mechanisms into the domesticated crop. Future studies are needed to assess how these observations may extend to other domesticated crops and other generalist pathogens.

**Introduction**

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

In contrast to specialist pathogens, generalist pathogens are virulent across a wide range of plant host species. Generalist pathogens potentially have less stringent co-evolution to specific hosts and their accompanying resistance mechanisms, because these pathogens can easily shift to new hosts in the environment. This allows generalist pathogens to evade the rapid evolution of new resistance mechanisms within specific hosts until they evolve to counter this new resistance. This niche-shifting ability may partially explain the observation that most natural resistance to generalist pathogens is highly polygenic, and the underlying plant genes for resistance are quantitative (Glazebrook 2005, Nomura, Melotto et al. 2005, Goss and Bergelson 2006, Rowe and Kliebenstein 2008, Barrett, Kniskern et al. 2009, Corwin, Copeland et al. 2016). Plant quantitative resistance genes to generalist pathogens include a broad array of direct defense genes, like those involved in secondary metabolite production, cell wall formation, and defense proteins (Zhang, Khan et al. 2002, Denby, Kumar et al. 2004, Zipfel, Robatzek et al. 2004, Ferrari, Galletti et al. 2007, Rowe and Kliebenstein 2008, Poland, Balint-Kurti et al. 2009, Corwin, Copeland et al. 2016). Importantly, these quantitative plant resistance loci do not alter resistance to all genotypes (isolates) of a pathogen but interact with the infecting pathogen’s genotype. For example, the ability of the *Arabidopsis* defense metabolite, camalexin, to provide resistance to *Botrytis cinerea* depends upon whether the specific isolate is sensitive or resistant to camalexin (Kliebenstein, Rowe et al. 2005, Stefanato, Abou‐Mansour et al. 2009) (Pedras and Ahiahonu , Pedras, Hossain et al.) and similarly *B. cinerea* virulence on tomato varies with the isolate’s ability to detoxify tomatine (Quidde, Osbourn et al. 1998, Quidde, Büttner et al.). In contrast to the polygenic nature of plant resistance to generalist pathogens, little is known about the genetic architecture of virulence within generalist pathogens, and how this is affected by genetic variation in the plant. There are no reported naturally variable large-effect virulence loci in generalist pathogens, suggesting that virulence in generalist pathogens is largely quantitative and polygenic. This potential for interaction between polygenic virulence in generalist pathogens and equally polygenic resistance in host plants 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 the domestication of crop plants. Domesticated plant varieties are typically more sensitive to specialist pathogens than their wild relatives (Smale 1996, Rosenthal and Dirzo 1997, Couch, Fudal et al. 2005, Dwivedi, Upadhyaya et al. 2008), and pathogens may evolve higher virulence on domesticated hosts (Stukenbrock and McDonald 2008). Further, domestication typically imposes a genetic bottleneck that reduces genetic diversity in the crop germplasm, including decreased availability of resistance alleles against specialist pathogens (Tanksley and McCouch 1997, Doebley, Gaut et al. 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, 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 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 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), 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 diverse 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 genomic sequence diversity spread across the genome (Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012, Atwell, Corwin et al. 2015, Atwell, Soltis et al. 2017). 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 diversity 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, 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* (12.3 SNP/kb) (Upadhyaya, Garnica et al. 2014). In addition to SNP diversity, the genomic sequencing showed that the species has a high level of recombination and genomic admixture, as if it were a randomly intermating population. 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, 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 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 quantified the interaction through lesion size in a detached leaf assay. 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, Kuwashima et al. 2000, Nicot, Moretti et al. 2002, Guimaraes, Chetelat et al. 2004, Ten Have, van Berloo et al. 2007, Finkers, Bai et al. 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. 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. In this study, we asked whether *B. cinerea* virulence was controlled by host variation, pathogen variation, or the interaction between them. Lesion size of *B. cinerea* is a quantitative trait that was controlled by plant domestication status, plant genotype and pathogen isolate. We looked for evidence of specialization within our generalist pathogen population. While our *B. cinerea* isolates appear to be generalists across domestication in *Solanum,* a subset of isolates are sensitive to tomato domestication. Finally, we aimed to identify the genetic basis of variation in *B. cinerea* virulence on domesticated and wild tomato. We conducted genome-wide association (GWA) in *B. cinerea* to identify pathogen loci where genetic variation leads to altered virulence across the host genotypes, including a specific test for loci that influence responses to crop 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 where allelic variation gives the isolates opposing responses to crop domestication. These pathogen loci could provide tools for understanding how domestication in tomato has influenced generalist pathogen resistance, to inform breeding efforts.

**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 isolated as single spores from natural infections of fruit and vegetable tissues collected in California and internationally (Atwell, Corwin et al. 2015, Zhang, Corwin et al. 2017). 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 minor allele frequency (MAF) 0.20 or greater, and less than 10% missing calls across the isolates (SNP calls in at least 82/ 91 isolates).

**Detached leaf assay**

To study the effect of genetic variation in host and pathogen on lesion formation, we infected detached leaves of 12 diverse tomato varieties with the above 97 *B. cinerea* isolates. We used a randomized complete block design for a total of 6 replicates across 2 experiments. In each experiment, this included a total of 10 plants per genotype randomized in 12 flats in 3 growth chambers. Each growth chamber block corresponded with a replicate of the detached leaf assay, such that growth chamber and replicate shared the same environmental block. At 6 weeks of age, we selected 5 leaves per plant (expanded leaves from second true leaf or 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, Kumar et al. 2004, Kliebenstein, Rowe et al. 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, 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 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; (Douglas Bates 2015)). Two of our 97 isolates that did not have replication across 2 experiments 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. We also calculated a domestication sensitivity phenotype, Sensitivity = (Domesticated lesion size – Wild lesion size) / Domesticated lesion size.

We used several methods to examine host specialization to tomato within *B. cinerea*. First, we split our *B. cinerea* population into isolates collected from tomato tissue vs. other hosts. We compared these groups by t-test for virulence on domesticated tomato genotypes, wild tomato genotypes, or all tomato genotypes. Next, we used a Wilcoxon signed-rank test to compare the rank order distribution of lesion sizes across paired tomato genotypes. To examine host specialization to tomato domestication within *B. cinerea*, we used a Wilcoxon signed-rank test to compare the rank order of lesion sizes across all domesticated vs. all wild tomato genotypes. Finally, we conducted single-isolate ANOVAs with FDR correction to identify isolates with a significant response to plant genotype or domestication status.

The model means and Sensitivity were used as the phenotypic input for GWA using bigRR, a heteroskedastic ridge regression method that incorporates SNP-specific shrinkage (Shen, Alam et al. 2013). This approach has previously had a high validation rate (Ober, Huang et al. 2015, Corwin, Copeland et al. 2016, Francisco, Joseph et al. 2016, Kooke, Kruijer et al. 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 and Churchill 1996, Shen, Alam et al. 2013, Corwin, Copeland et al. 2016). SNPs were annotated using SNPdat (Doran and Creevey 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 and van Kan 2012)). Functional annotations are based on the T4 gene models for genomic DNA (http://www.broadinstitute.org, *B. cinerea*; (Staats and van Kan 2012)). Additional genes of interest, based on a broad literature search of known virulence loci, were taken from NCBI (https://www.ncbi.nlm.nih.gov/) and included by mapping sequence to the T4 reference using MUMmer v3.0 (Kurtz, Phillippy et al. 2004). We used the program InterProScan within BLAST2GO for functional gene ontology (GO) annotation of the gene models (http://www.blast2go.com).

To predict expected overlap of significant SNPs across plant genotypes, we used the average number of significant SNPs per each of the 12 plant genotypes (14,000 SNPs) and calculated expected overlap between those 12 lists using binomial coefficients.

**Results**

**Experimental Design**

To measure 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 wild and domesticated tomato genotypes. We compared domesticated and closely related wild tomatoes 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, Spooner et al. 2008, Müller, Wijnen et al. 2016). Our previously collected *B. cinerea* sample includes 97 isolates obtained from various eudicot plant hosts, including tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2, Supersteak)(Atwell, Soltis et al.). 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).

**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 becomes visible surrounding the location of the spore droplet, but no expansion is 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 observed a mean lesion size of 0.67 cm2 across the full experiment, with 0.94 CV across the full isolate population on all tomato genotypes. Individual isolates were highly variable in their lesion size across tomato genotypes(Figure 2), with mean lesion size per isolate of 0.14 cm2 to 1.29 cm2, and individual isolate coefficient of variation CV from 0.51 to 1.68 across all observations on all tomato genotypes. A subset of these isolates are highly virulent on tomato (mean lesion size > 1.05 cm2, Figure 2C), and a subset can be considered saprophytic (mean lesion size < 0.3 cm2, Figure 2D).

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

To measure the relative contribution of genetic diversity in the plant and the pathogen to variation in the virulence/ resistance phenotype, we used a multiple linear regression model (R Development Core Team 2008). 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 total variance for lesion size, and showed that genetic variation within both the host plant and the pathogen had significant effects on lesion growth, with pathogen isolate diversity explaining 3.5 fold more variance than plant genotype, 45.8% of total genetic variance for pathogen isolate vs. 13.2% for plant genotype (Table 1 and Figure 2A). 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 (3.5% of total genetic variance, Table 1). 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 (33.8% of total genetic variance, Table 1). The lack of significance for this term in face of the large fraction of variance may be due to the vast degrees of freedom in this term (Table 1). 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 (Smale 1996, Rosenthal and Dirzo 1997, Couch, Fudal et al. 2005, Dwivedi, Upadhyaya et al. 2008, Stukenbrock and McDonald 2008). In our analysis, we identified a significant increase, 18%, in the resistance of wild tomato in comparison to domesticated tomato across the population of *B. cinerea* isolates (Figure 3, Table 1). However, this domestication effect was not the dominant source of variation, as genetic variation within the domesticated and wild genotypes contributed 3.8 fold more variation in resistance than domestication alone (Table 1). So while we did observe the expected decreased resistance in domesticated tomato, domestication was a minor player in controlling lesion size variation, with most of the plant genetic signature coming from variation within both the wild and domesticated tomato species.

In addition to altering trait means, domestication commonly decreases genetic variation in comparison to wild germplasm due to bottlenecks, including for tomato (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Bai and Lindhout 2007). This decreased genetic variation should also limit phenotypic variation, including disease phenotypes. Interestingly in this tomato population, we did not observe reduced variation in lesion size in the wild tomato. Indeed, 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 (standard deviation = 0.68 cm2) versus 1.76 cm2 variation on wild tomato (standard deviation = 0.58 cm2). Additionally, the wild and domesticated tomato genotypes showed statistically similar variation in resistance (F-test, F96,96=1.39, p=0.11)(Figure 4). Overall, there is a slight domestication impact on average resistance to *B. cinerea*, 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 even between individuals. 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 on domesticated tomato (t-test; t=1.10, n = 97, p=0.33), wild tomato (t-test; t=1.09, n = 97, p=0.33) or across all tomato genotypes (t-test; n = 97, p=0.14) (Figure 2E). In fact, one isolate collected from tomato tissue (KGB1) was within the 10 least-virulent isolates and another (Triple3) was within the 10 most-virulent isolates (Figure 2E). This demonstrated significant genetic variation in virulence across the *B. cinerea* isolates, and that this collection of *B. cinerea* isolates from tomato do not display a strong host-specificity for tomato (Martinez, Blancard et al. 2003, Ma and Michailides 2005, Rowe and Kliebenstein 2007, Samuel, Veloukas et al. 2012).

**Pathogen Specialization to Host 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 (Rowe and Kliebenstein 2007, Kretschmer and Hahn 2008, Corwin, Subedy et al. 2016). A visual analysis of the data suggested an interaction between the genomes of *B. cinerea* and tomato (Figure 2). 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 these terms (Table 1). 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 by plant genotype interaction term with 940 degrees of freedom (Table 1). To assess these 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 significantly changes between pairs of tomato genotypes. This showed that when using the full isolate population, the rank performance of the isolates does significantly vary between host genotypes. When comparing mean lesion size between paired plant genotypes, 58% (38 out of 66) of tomato accession pairs had significantly different ranking of the isolates (Wilcoxon signed-rank test with FDR-correction, Table 2, Figure 5). A significant p-value indicates that the two host genotypes show evidence for different virulence interactions with the population of *B*. *cinerea* isolates, providing evidence for host x pathogen genotypic interactions. This pattern was consistent across domesticated host pairs, wild host pairs, or between-species host pairs (Wilcoxon signed-rank test with FDR-correction, Table 2). This suggests that the population of *B. cinerea* does display differential responses to the tomato genetic variation.

To focus on whether specific *B. cinerea* isolates may be sensitive to domestication, we applied a Wilcoxon and ANOVA approach. Overall, most isolates (78/97, 80%) are more virulent on domesticated than wild tomato (Figure 4). The Wilcoxon signed-rank test, to compare the rank of mean lesion size of all the *B. cinerea* isolates on wild versus domesticated tomato, was significant (Wilcoxon signed-rank test, W = 5946, p-value = 0.002) (Figure 4). To identify the pathogen genotypes most sensitive to domestication, we conducted single-isolate ANOVAs including the fixed effects of plant, domestication, and experiment, and found two isolates with a significant effect of domestication on lesion size (p < 0.05, FDR corrected) (Figure 2F), both of which are more virulent on domesticated tomato. These included one of the highly virulent isolates (Fd2), and one of the largely saprophytic isolates (Rose), which suggests that isolate virulence level on tomato does not predict *B. cinerea* genetic response to tomato domestication. Both of these isolates were more virulent on domesticated than on wild tomato. These results suggest that this *B. cinerea* population contains two highly domestication-sensitive isolates which are more virulent on domesticated tomato, and 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 interacted with tomato domestication (Table 1). This suggests that there is genetic variation within the pathogen in which some alleles enhance and other alleles decrease virulence depending upon the plant’s genotype. To identify variable pathogen genes controlling differential virulence across plant genotypes, 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, Alam et al. 2013, Corwin, Copeland et al. 2016, Corwin, Subedy et al. 2016, Francisco, Joseph et al. 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% permutation threshold). The estimated effect size of each individual SNP was small (Figure 6).

Only a small subset of these *B. cinerea* SNPs were linked to virulence on all the tomato genotypes. However, we identified genes linked to all tomato genotypes at a higher rate, as SNPs associated with different host genotypes were located within a single gene window. We found five *B. cinerea* SNPs significantly linked to altered lesion size on all 12 tomato accessions (Figure 6B). 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 7A). To change from a SNP-by-SNP focus to a gene-centric focus, we classified a gene as significantly associated if there was 1 SNP linked to a trait using a 2kbp window surrounding the start and stop codon for a given gene. This found 6 genes linked to differential virulence in all 12 tomato accessions (Figure 7b, Table S1), as in some cases multiple accession-specific SNPs (significant in <12 tomato accessions) were linked to a single gene. A further 233 genes were linked to differential virulence on 7 to 11 tomato accessions (Figure 7b, Table S1). Of the 6 genes with SNPs significantly associated with B. cinerea virulence, two are heterokaryon incompatibility loci (Bcin01g10020 , BcT4\_2485 (no BCIN)), one is a major facilitator superfamily gene, and the remaining 3 are enzymes (peptidase dimerization Bcin01g10130, pectinesterase Bcin14g00860 , protein kinase Bcin15g04110 ). Four of those same functional annotations are significantly overrepresented due to one or more genes associated with the 12 plant genotypes, including heterokaryon incompatibility, pectinesterase, peptidase dimerization, and protein kinase.To visualize the SNP effects across a single gene and look for evidence of multiple haplotypes, we focused on a single pectinesterase gene (BcT4\_6001, Bcin14g00870) that is significantly associated with virulence variation across all of the tomato genotypes (Figure 8). This showed that the effect of SNPs across this gene vary in effect direction depending on tomato host genotype (Figure 8a), and that multiple haplotype blocks contribute to the association of this gene to the virulence phenotype (Figure 8b). 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.

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

The identification of two isolates that distinctly respond to tomato domestication suggests that there is natural genetic variation in *B. cinerea* that is affected by tomato domestication. To directly map *B. cinerea* genes that control differential virulence on wild versus domestic 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 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 6, Figure 9). 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 9). To begin querying the underlying gene functions for these various *B. cinerea* loci, we called genes as significant if there was one SNP within 2kb of that gene (Figure 9C). Using all 1251 genes linked to domestication phenotypes in a functional enrichment analysis found only 22 biological functions that were significantly overrepresented (Fisher exact test, p<0.05, Table S1) when compared to the whole-genome annotation. The functions overrepresented for domestication traits include 8 classes of enzymes and two transporters (Table S1). Eight of these are uniquely overrepresented in *B. cinerea* growth on wild tomato genotypes, and eight functions are overrepresented only for domestication-sensitivity genes. Among the sensitivity functions is indoleamine, which may degrade tryptophan in tomato, and phosphodiesterase, a function that has previously been associated with *B. cinerea* virulence through the cAMP signaling pathway (Harren, Brandhoff et al. 2013). Thus, the genetic architecture of how *B. cinerea* is influenced by the genetic variation imparted during 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 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). Previous studies on 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 within tomato influenced the interaction at the level of the pathogen population and individual genes in the pathogen. *B. cinerea* virulence on tomato, as measured by lesion size, was significantly affected by pathogen isolate, host genotype, and domestication status (Table 1). 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 domesticated tomato accessions having similar variance in resistance (Table 1, Figure 3). 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 2) (Giraud, Fortini et al. 1999, Martinez, Blancard et al. 2003, Ma and Michailides 2005). GWA mapping within the pathogen showed that the genetics underlying *B. cinerea* virulence on tomato are highly quantitative, and vary across tomato genotypes and domestication status (Figure 7, Figure 9). This analysis identified a small subset of pathogen genes whose variation contributes to differential virulence on most of the hosts tested, and a set of pathogen genes whose variation is responsive to tomato domestication (Table S1).

**Domestication and altered pathogen virulence genetics**

These results provide evidence of a mild tomato domestication effect on resistance to the generalist pathogen, *B. cinerea.* We measured an 18% increase in susceptibility across domesticated varieties, but this represents less than 1% of the total variance of *B. cinerea* lesion size on tomato (Table 1). As such, domestication status alone is a poor predictor of a specific tomato host’s resistance to infection by *B. cinerea*. This suggests that while tomato domestication does affect this plant-pathogen interaction, it is not the primary factor defining the measured trait. 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 (Figure 2). If a study relies on one or a few isolates, it could obtain a falsely high or falsely low estimation of how host domestication influences pathogen resistance. This shows the need to utilize a population of *B. cinerea* to understand the factors contributing to *B. cinerea* virulence and how this is altered by crop domestication.

In biotrophic pathogens, host domestication has decreased the diversity of resistance alleles because they are lost in the domestication bottleneck as found for specialist pathogens (Tanksley and McCouch 1997, Doebley, Gaut et al. 2006, Hyten, Song et al. 2006, Chaudhary 2013). Surprisingly, we did not find evidence for a domestication bottleneck in the phenotypic resistance to *B. cinerea* (Figure 3, Figure 4). This is in contrast to genomic studies that explicitly show a genotypic bottleneck within tomato domestication (Miller and Tanksley 1990, Koenig, Jiménez-Gómez et al. 2013). 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 decrease 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 6a). Importantly, the tomato host accession greatly influenced which *B. cinerea* loci were significantly associated to lesion size (Figure 7). Thus, it possible that there is specialization at the gene level, in which different alleles within the pathogen link to differential virulence on specific host genotypes (Giraud, Fortini et al. 1999, Rowe and Kliebenstein 2007, Blanco-Ulate, Morales-Cruz et al. 2014). This polygenic architecture of virulence is distinctly different from specialist pathogens that often have one or a few large effect genes that control virulence (Keen 1992, De Feyter, Yang et al. 1993, Abramovitch and Martin 2004, Boyd, Ridout et al. 2013, Vleeshouwers and Oliver 2014). Further studies are needed to compare how the host plant species may affect this image of genetic variation in virulence.

These results indicate some particular challenges for breeding durable resistance to *B. cinerea* and possibly other generalist pathogens. The highly polygenic variation in virulence combined with genomic sequencing, showing that this pathogen is an inter-breeding population, suggests that the pathogen is actively blending a large collection of polymorphic virulence loci (Rowe and Kliebenstein 2007, Fekete, Fekete et al. 2012, Atwell, Corwin et al. 2015, Atwell, Soltis et al. 2017). 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 virulence 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 (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. However, the lack of 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.

**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. Several of the functions we identified suggest new pathogen virulence loci. Two of the loci associated with virulence on 12 plant host genotypes are heterokaryon incompatibility proteins (Bcin01g10020, BcT4\_2485 (no BCIN)). Variation at these loci may determine the ability of isolates to undergo asexual recombination.

Additional loci may increase *B. cinerea* virulence on tomato through metabolic shifts. Through analysis of loci contributing to virulence on all 12 host genotypes, we identified a pectinesterase (Bcin14g00860), a peptidase dimerization domain (Bcin01g10130), and a protein kinase (Bcin15g04110) (Table S1).

The mechanisms of quantitative virulence identified in this study are in contrast to previously-described qualitative virulence loci. Using specific *a priori* gene searches, we did not identify any other known fungal PAMPs, i.e. mannans, glycans or glycolipid genes, as loci contributing to variation in virulence across tomato accessions (Romani 2004, Hématy, Cherk et al. 2009, Gust 2015, Corwin, Copeland et al. 2016, Corwin, Subedy et al. 2016). We also did not identify known virulence loci such as NEPs, VELVET or polygalacturonases (ten Have, Mulder et al. 1998, De Lorenzo and Ferrari 2002, Choquer, Fournier et al. 2007, Schumacher, Pradier et al. 2012, Yang, Chen et al. 2013). All of these genes did have SNPs within the analysis, suggesting that they could have been found via the GWA. It is possible that the size of the population was simply not powerful enough to identify these loci, but this does show that we are able to identify new virulence loci within *B. cinerea* that likely have new resistance/sensitivity targets in the plant.

**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 1. 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 | % total variance | % genetic variance | SS | F value | DF | p |
| Isolate | 10.2 | 45.8 | 256.6 | 13.54 | 94 | **<2e-16** |
| Domestication | 0.8 | 3.5 | 19.45 | 96.46 | 1 | **<2e-16** |
| Domest/Plant | 2.9 | 13.2 | 73.67 | 36.54 | 10 | **<2e-16** |
| Iso:Domest | 0.8 | 3.7 | 20.67 | 1.091 | 94 | 0.260 |
| Iso:Domest/Plant | 7.5 | 33.8 | 189.5 | 0.9838 | 940 | 0.623 |
| Experiment | 21.7 |  | 545.7 | 2707 | 1 | **<2e-16** |
| Exp/Block | 8.0 |  | 201.0 | 249.3 | 4 | **<2e-16** |
| Exp:Iso | 6.0 |  | 152.2 | 8.028 | 94 | **<2e-16** |
| Exp:Domest | 0.03 |  | 0.83 | 4.095 | 1 | 0.043 |
| Exp:Domest/Plant | 1.9 |  | 47.43 | 23.53 | 10 | **<2e-16** |
| Residuals | 40.1 |  | 1009 |  |  |  |

**Table 2. Rank order shifts of 97 *B. cinerea* isolates by lesion area across all of the tomato accessions.**

Wilcoxon signed-rank test comparing 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 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 |  | 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 |
| Domesticated | LA2706 | **<0.001** | **0.002** | **<0.001** | **<0.001** | NS | **0.003** |  | 6071 | 4564 | 3785 | 6716 | 5794 |
| LA3008 | **0.003** | NS | NS | *0.607* | **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 1. *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 2. Highlighted variance of diversity in *B. cinerea* x tomato interactions.**

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 3. 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 4. 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). 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 5. 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 rank order of isolates by lesion size (Wilcoxon signed-rank test with FDR-correction, p < 7.18e-17, Table 2).

**Figure 6. 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 7. 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 8. Host specificity of significant SNPs linked to the gene BcT4\_4591 (Bcin02g06830).**

A) SNPs with effects estimates above the 99% permutation threshold are colored by trait (plant phenotype in which the effect was estimated). BcT4\_4591 (Bcin02g06830) 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.

B) Linkage disequilibrium plot, including all pairwise comparisons of SNPs in the 2kb region surrounding BcT4\_4591. The color scheme for each SNP pair is D'/LOD: white if LOD <2 and D’ <1, bright red for LOD ≥2 and D’=1, intermediate shades for LOD≥2 and D’<1.

**Figure 9. 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 and using calculated Sensitivity. 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.

**References**

Abramovitch, R. B. and G. B. Martin (2004). "Strategies used by bacterial pathogens to suppress plant defenses." Current opinion in plant biology **7**(4): 356-364.

AbuQamar, S., M.-F. Chai, H. Luo, F. Song and T. Mengiste (2008). "Tomato protein kinase 1b mediates signaling of plant responses to necrotrophic fungi and insect herbivory." The Plant Cell **20**(7): 1964-1983.

Atwell, S., J. Corwin, N. Soltis, A. Subedy, K. Denby and D. J. Kliebenstein (2015). "Whole genome resequencing of Botrytis cinerea isolates identifies high levels of standing diversity." Frontiers in microbiology **6**: 996.

Atwell, S., N. Soltis and D. J. Kliebenstein (2017). "Genetic Diversity in 97 Botrytis cinerea Isolates." in prep.

Baccelli, I. (2014). "Cerato-platanin family proteins: one function for multiple biological roles?" Frontiers in plant science **5**.

Bai, Y. and P. Lindhout (2007). "Domestication and breeding of tomatoes: what have we gained and what can we gain in the future?" Annals of botany **100**(5): 1085-1094.

Barrett, L. G. and M. Heil (2012). "Unifying concepts and mechanisms in the specificity of plant–enemy interactions." Trends in plant science **17**(5): 282-292.

Barrett, L. G., J. M. Kniskern, N. Bodenhausen, W. Zhang and J. Bergelson (2009). "Continua of specificity and virulence in plant host–pathogen interactions: causes and consequences." New Phytologist **183**(3): 513-529.

Bergougnoux, V. (2014). "The history of tomato: from domestication to biopharming." Biotechnology advances **32**(1): 170-189.

Bhardwaj, V., S. Meier, L. N. Petersen, R. A. Ingle and L. C. Roden (2011). "Defence responses of Arabidopsis thaliana to infection by Pseudomonas syringae are regulated by the circadian clock." PloS one **6**(10): e26968.

Bittel, P. and S. Robatzek (2007). "Microbe-associated molecular patterns (MAMPs) probe plant immunity." Current opinion in plant biology **10**(4): 335-341.

Blanco-Ulate, B., A. Morales-Cruz, K. C. Amrine, J. M. Labavitch, A. L. Powell and D. Cantu (2014). "Genome-wide transcriptional profiling of Botrytis cinerea genes targeting plant cell walls during infections of different hosts." Frontiers in plant science **5**.

Boller, T. and S. Y. He (2009). "Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens." Science **324**(5928): 742-744.

Boyd, L. A., C. Ridout, D. M. O'Sullivan, J. E. Leach and H. Leung (2013). "Plant–pathogen interactions: disease resistance in modern agriculture." Trends in genetics **29**(4): 233-240.

Chaudhary, B. (2013). "Plant domestication and resistance to herbivory." International journal of plant genomics **2013**.

Choquer, M., E. Fournier, C. Kunz, C. Levis, J.-M. Pradier, A. Simon and M. Viaud (2007). "Botrytis cinerea virulence factors: new insights into a necrotrophic and polyphageous pathogen." FEMS microbiology letters **277**(1): 1-10.

Corwin, J. A., D. Copeland, J. Feusier, A. Subedy, R. Eshbaugh, C. Palmer, J. Maloof and D. J. Kliebenstein (2016). "The quantitative basis of the Arabidopsis innate immune system to endemic pathogens depends on pathogen genetics." PLoS genetics **12**(2): e1005789.

Corwin, J. A., D. Copeland, J. Feusier, A. Subedy, R. Eshbaugh, C. Palmer, J. Maloof and D. J. Kliebenstein (2016). "The quantitative basis of the Arabidopsis innate immune system to endemic pathogens depends on pathogen genetics." PLoS Genet **12**(2): e1005789.

Corwin, J. A., A. Subedy, R. Eshbaugh and D. J. Kliebenstein (2016). "Expansive phenotypic landscape of Botrytis cinerea shows differential contribution of genetic diversity and plasticity." Molecular Plant-Microbe Interactions **29**(4): 287-298.

Couch, B. C., I. Fudal, M.-H. Lebrun, D. Tharreau, B. Valent, P. Van Kim, J.-L. Nottéghem and L. M. Kohn (2005). "Origins of host-specific populations of the blast pathogen Magnaporthe oryzae in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice." Genetics **170**(2): 613-630.

Dalmais, B., J. Schumacher, J. Moraga, P. Le Pecheur, B. Tudzynski, I. G. Collado and M. Viaud (2011). "The Botrytis cinerea phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial." Molecular plant pathology **12**(6): 564-579.

Dangl, J. L. and J. D. Jones (2001). "Plant pathogens and integrated defence responses to infection." nature **411**(6839): 826-833.

De Feyter, R., Y. Yang and D. W. Gabriel (1993). "Gene-for-genes interactions between cotton R genes and Xanthomonas campestris pv. malvacearum avr genes." Molecular plant-microbe interactions: MPMI **6**(2): 225-237.

De Lorenzo, G. and S. Ferrari (2002). "Polygalacturonase-inhibiting proteins in defense against phytopathogenic fungi." Current opinion in plant biology **5**(4): 295-299.

Dean, R., J. A. Van Kan, Z. A. Pretorius, K. E. Hammond‐Kosack, A. Di Pietro, P. D. Spanu, J. J. Rudd, M. Dickman, R. Kahmann and J. Ellis (2012). "The Top 10 fungal pathogens in molecular plant pathology." Molecular plant pathology **13**(4): 414-430.

Deighton, N., I. Muckenschnabel, A. J. Colmenares, I. G. Collado and B. Williamson (2001). "Botrydial is produced in plant tissues infected by Botrytis cinerea." Phytochemistry **57**(5): 689-692.

Denby, K. J., P. Kumar and D. J. Kliebenstein (2004). "Identification of Botrytis cinerea susceptibility loci in Arabidopsis thaliana." The Plant Journal **38**(3): 473-486.

Desjardins, A. E. and T. M. Hohn (1997). "Mycotoxins in plant pathogenesis." Molecular Plant-Microbe Interactions **10**(2): 147-152.

Desjardins, C. A., K. A. Cohen, V. Munsamy, T. Abeel, K. Maharaj, B. J. Walker, T. P. Shea, D. V. Almeida, A. L. Manson and A. Salazar (2016). "Genomic and functional analyses of Mycobacterium tuberculosis strains implicate ald in D-cycloserine resistance." Nature genetics **48**(5): 544-551.

Dıaz, J., A. ten Have and J. A. van Kan (2002). "The role of ethylene and wound signaling in resistance of tomato to Botrytis cinerea." Plant physiology **129**(3): 1341-1351.

Dodds, P. N. and J. P. Rathjen (2010). "Plant immunity: towards an integrated view of plant–pathogen interactions." Nature Reviews Genetics **11**(8): 539-548.

Doebley, J. F., B. S. Gaut and B. D. Smith (2006). "The molecular genetics of crop domestication." Cell **127**(7): 1309-1321.

Doerge, R. W. and G. A. Churchill (1996). "Permutation tests for multiple loci affecting a quantitative character." Genetics **142**(1): 285-294.

Doran, A. G. and C. J. Creevey (2013). "Snpdat: Easy and rapid annotation of results from de novo snp discovery projects for model and non-model organisms." BMC bioinformatics **14**(1): 45.

Douglas Bates, M. M., Ben Bolker, Steve Walker (2015). "Fitting Linear Mixed-Effects Models Using lme4." Journal of Statistical Software **67**(1): 1-48.

Dwivedi, S. L., H. D. Upadhyaya, H. T. Stalker, M. W. Blair, D. J. Bertioli, S. Nielen and R. Ortiz (2008). "Enhancing crop gene pools with beneficial traits using wild relatives." Plant Breeding Reviews **30**: 179.

Egashira, H., A. Kuwashima, H. Ishiguro, K. Fukushima, T. Kaya and S. Imanishi (2000). "Screening of wild accessions resistant to gray mold (Botrytis cinerea Pers.) in Lycopersicon." Acta physiologiae plantarum **22**(3): 324-326.

Elad, Y., B. Williamson, P. Tudzynski and N. Delen (2007). Botrytis spp. and diseases they cause in agricultural systems–an introduction. Botrytis: Biology, pathology and control, Springer**:** 1-8.

Failmezger, H., Y. Yuan, O. Rueda, F. Markowetz and M. H. Failmezger (2012). "CRImage: CRImage a package to classify cells and calculate tumour cellularity." R package version 1.24.0.

Farhat, M. R., B. J. Shapiro, K. J. Kieser, R. Sultana, K. R. Jacobson, T. C. Victor, R. M. Warren, E. M. Streicher, A. Calver and A. Sloutsky (2013). "Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis." Nature genetics **45**(10): 1183-1189.

Fekete, É., E. Fekete, L. Irinyi, L. Karaffa, M. Árnyasi, M. Asadollahi and E. Sándor (2012). "Genetic diversity of a Botrytis cinerea cryptic species complex in Hungary." Microbiological Research **167**(5): 283-291.

Ferrari, S., R. Galletti, C. Denoux, G. De Lorenzo, F. M. Ausubel and J. Dewdney (2007). "Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3." Plant physiology **144**(1): 367-379.

Ferrari, S., J. M. Plotnikova, G. De Lorenzo and F. M. Ausubel (2003). "Arabidopsis local resistance to Botrytis cinerea involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4." The Plant Journal **35**(2): 193-205.

Fillinger, S. and Y. Elad (2015). Botrytis-the Fungus, the Pathogen and Its Management in Agricultural Systems, Springer.

Finkers, R., Y. Bai, P. van den Berg, R. van Berloo, F. Meijer-Dekens, A. Ten Have, J. van Kan, P. Lindhout and A. W. van Heusden (2008). "Quantitative resistance to Botrytis cinerea from Solanum neorickii." Euphytica **159**(1-2): 83-92.

Finkers, R., A. W. van Heusden, F. Meijer-Dekens, J. A. van Kan, P. Maris and P. Lindhout (2007). "The construction of a Solanum habrochaites LYC4 introgression line population and the identification of QTLs for resistance to Botrytis cinerea." Theoretical and Applied Genetics **114**(6): 1071-1080.

Francisco, M., B. Joseph, H. Caligagan, B. Li, J. A. Corwin, C. Lin, R. E. Kerwin, M. Burow and D. J. Kliebenstein (2016). "Genome wide association mapping in Arabidopsis thaliana identifies novel genes involved in linking allyl glucosinolate to altered biomass and defense." Frontiers in plant science **7**.

Frías, M., N. Brito and C. González (2013). "The Botrytis cinerea cerato‐platanin BcSpl1 is a potent inducer of systemic acquired resistance (SAR) in tobacco and generates a wave of salicylic acid expanding from the site of application." Molecular plant pathology **14**(2): 191-196.

Gaderer, R., K. Bonazza and V. Seidl-Seiboth (2014). "Cerato-platanins: a fungal protein family with intriguing properties and application potential." Applied microbiology and biotechnology **98**(11): 4795-4803.

Giraud, T., D. Fortini, C. Levis, C. Lamarque, P. Leroux, K. LoBuglio and Y. Brygoo (1999). "Two sibling species of the Botrytis cinerea complex, transposa and vacuma, are found in sympatry on numerous host plants." Phytopathology **89**(10): 967-973.

Glazebrook, J. (2005). "Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens." Annu. Rev. Phytopathol. **43**: 205-227.

Gonçalves, I. R., S. Brouillet, M.-C. Soulié, S. Gribaldo, C. Sirven, N. Charron, M. Boccara and M. Choquer (2016). "Genome-wide analyses of chitin synthases identify horizontal gene transfers towards bacteria and allow a robust and unifying classification into fungi." BMC evolutionary biology **16**(1): 252.

Goss, E. M. and J. Bergelson (2006). "Variation in resistance and virulence in the interaction between Arabidopsis thaliana and a bacterial pathogen." Evolution **60**(8): 1562-1573.

Guimaraes, R. L., R. T. Chetelat and H. U. Stotz (2004). "Resistance to Botrytis cinerea in Solanum lycopersicoides is dominant in hybrids with tomato, and involves induced hyphal death." European journal of plant pathology **110**(1): 13-23.

Gust, A. A. (2015). "Peptidoglycan perception in plants." PLoS pathogens **11**(12): e1005275.

Hacquard, S., B. Kracher, T. Maekawa, S. Vernaldi, P. Schulze-Lefert and E. V. L. van Themaat (2013). "Mosaic genome structure of the barley powdery mildew pathogen and conservation of transcriptional programs in divergent hosts." Proceedings of the National Academy of Sciences **110**(24): E2219-E2228.

Hahn, M. (2014). "The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study." Journal of chemical biology **7**(4): 133-141.

Harren, K., B. Brandhoff, M. Knödler and B. Tudzynski (2013). "The high-affinity phosphodiesterase BcPde2 has impact on growth, differentiation and virulence of the phytopathogenic ascomycete Botrytis cinerea." PLOS one **8**(11): e78525.

Hématy, K., C. Cherk and S. Somerville (2009). "Host–pathogen warfare at the plant cell wall." Current opinion in plant biology **12**(4): 406-413.

Hevia, M. A., P. Canessa, H. Müller-Esparza and L. F. Larrondo (2015). "A circadian oscillator in the fungus Botrytis cinerea regulates virulence when infecting Arabidopsis thaliana." Proceedings of the National Academy of Sciences **112**(28): 8744-8749.

Hyten, D. L., Q. Song, Y. Zhu, I.-Y. Choi, R. L. Nelson, J. M. Costa, J. E. Specht, R. C. Shoemaker and P. B. Cregan (2006). "Impacts of genetic bottlenecks on soybean genome diversity." Proceedings of the National Academy of Sciences **103**(45): 16666-16671.

Jones, J. D. and J. L. Dangl (2006). "The plant immune system." Nature **444**(7117): 323-329.

Katan, T. (1999). "Current status of vegetative compatibility groups in Fusarium oxysporum." Phytoparasitica **27**(1): 51-64.

Keen, N. (1992). "The molecular biology of disease resistance." Plant molecular biology **19**(1): 109-122.

Kliebenstein, D. J., H. C. Rowe and K. J. Denby (2005). "Secondary metabolites influence Arabidopsis/Botrytis interactions: variation in host production and pathogen sensitivity." The Plant Journal **44**(1): 25-36.

Koenig, D., J. M. Jiménez-Gómez, S. Kimura, D. Fulop, D. H. Chitwood, L. R. Headland, R. Kumar, M. F. Covington, U. K. Devisetty and A. V. Tat (2013). "Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato." Proceedings of the National Academy of Sciences **110**(28): E2655-E2662.

Kooke, R., W. Kruijer, R. Bours, F. F. Becker, A. Kuhn, J. Buntjer, T. Doeswijk, J. Guerra, H. J. Bouwmeester and D. Vreugdenhil (2016). "Genome-wide association mapping and genomic prediction elucidate the genetic architecture of morphological traits in Arabidopsis thaliana." Plant Physiology: pp. 00997.02015.

Kover, P. X. and B. A. Schaal (2002). "Genetic variation for disease resistance and tolerance among Arabidopsis thaliana accessions." Proceedings of the National Academy of Sciences **99**(17): 11270-11274.

Kretschmer, M. and M. Hahn (2008). "Fungicide resistance and genetic diversity of Botrytis cinerea isolates from a vineyard in Germany." Journal of Plant Diseases and Protection: 214-219.

Kurtz, S., A. Phillippy, A. L. Delcher, M. Smoot, M. Shumway, C. Antonescu and S. L. Salzberg (2004). "Versatile and open software for comparing large genomes." Genome biology **5**(2): R12.

Liu, B., Y.-B. Hong, Y.-F. Zhang, X.-H. Li, L. Huang, H.-J. Zhang, D.-Y. Li and F.-M. Song (2014). "Tomato WRKY transcriptional factor SlDRW1 is required for disease resistance against Botrytis cinerea and tolerance to oxidative stress." Plant Science **227**: 145-156.

Loxdale, H. D., G. Lushai and J. A. Harvey (2011). "The evolutionary improbability of ‘generalism’in nature, with special reference to insects." Biological Journal of the Linnean Society **103**(1): 1-18.

Ma, Z. and T. J. Michailides (2005). "Genetic structure of Botrytis cinerea populations from different host plants in California." Plant disease **89**(10): 1083-1089.

Martinez, F., D. Blancard, P. Lecomte, C. Levis, B. Dubos and M. Fermaud (2003). "Phenotypic differences between vacuma and transposa subpopulations of Botrytis cinerea." European Journal of Plant Pathology **109**(5): 479-488.

Miller, J. and S. Tanksley (1990). "RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon." TAG Theoretical and Applied Genetics **80**(4): 437-448.

Müller, N. A., C. L. Wijnen, A. Srinivasan, M. Ryngajllo, I. Ofner, T. Lin, A. Ranjan, D. West, J. N. Maloof and N. R. Sinha (2016). "Domestication selected for deceleration of the circadian clock in cultivated tomato." Nature genetics **48**(1): 89-93.

Nicot, P., A. Moretti, C. Romiti, M. Bardin, C. Caranta and H. Ferriere (2002). "Differences in susceptibility of pruning wounds and leaves to infection by Botrytis cinerea among wild tomato accessions." TGC Report **52**: 24-26.

Nicot, P. C. and A. Baille (1996). Integrated control of Botrytis cinerea on greenhouse tomatoes. Aerial Plant Surface Microbiology, Springer**:** 169-189.

Nomura, K., M. Melotto and S.-Y. He (2005). "Suppression of host defense in compatible plant–Pseudomonas syringae interactions." Current opinion in plant biology **8**(4): 361-368.

Ober, U., W. Huang, M. Magwire, M. Schlather, H. Simianer and T. F. Mackay (2015). "Accounting for genetic architecture improves sequence based genomic prediction for a Drosophila fitness trait." PLoS One **10**(5): e0126880.

Ormond, E. L., A. P. Thomas, P. J. Pugh, J. K. Pell and H. E. Roy (2010). "A fungal pathogen in time and space: the population dynamics of Beauveria bassiana in a conifer forest." FEMS microbiology ecology **74**(1): 146-154.

Panthee, D. R. and F. Chen (2010). "Genomics of fungal disease resistance in tomato." Current genomics **11**(1): 30-39.

Parlevliet, J. E. (2002). "Durability of resistance against fungal, bacterial and viral pathogens; present situation." Euphytica **124**(2): 147-156.

Pau, G., F. Fuchs, O. Sklyar, M. Boutros and W. Huber (2010). "EBImage—an R package for image processing with applications to cellular phenotypes." Bioinformatics **26**(7): 979-981.

Pazzagli, L., V. Seidl-Seiboth, M. Barsottini, W. A. Vargas, A. Scala and P. K. Mukherjee (2014). "Cerato-platanins: elicitors and effectors." Plant Science **228**: 79-87.

Pedras, M. S. C. and P. W. Ahiahonu (2005). "Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi." Phytochemistry **66**(4): 391-411.

Pedras, M. S. C., S. Hossain and R. B. Snitynsky (2011). "Detoxification of cruciferous phytoalexins in Botrytis cinerea: Spontaneous dimerization of a camalexin metabolite." Phytochemistry **72**(2): 199-206.

Peralta, I., D. Spooner and S. Knapp (2008). "The taxonomy of tomatoes: a revision of wild tomatoes (Solanum section Lycopersicon) and their outgroup relatives in sections Juglandifolium and Lycopersicoides." Syst Bot Monogr **84**: 1-186.

Persoons, A., E. Morin, C. Delaruelle, T. Payen, F. Halkett, P. Frey, S. De Mita and S. Duplessis (2014). "Patterns of genomic variation in the poplar rust fungus Melampsora larici-populina identify pathogenesis-related factors." Frontiers in plant science **5**.

Pieterse, C. M., D. Van der Does, C. Zamioudis, A. Leon-Reyes and S. C. Van Wees (2012). "Hormonal modulation of plant immunity." Annual review of cell and developmental biology **28**: 489-521.

Poland, J. A., P. J. Balint-Kurti, R. J. Wisser, R. C. Pratt and R. J. Nelson (2009). "Shades of gray: the world of quantitative disease resistance." Trends in plant science **14**(1): 21-29.

Power, R. A., J. Parkhill and T. de Oliveira (2017). "Microbial genome-wide association studies: lessons from human GWAS." Nature Reviews Genetics **18**(1): 41-50.

Quidde, T., P. Büttner and P. Tudzynski (1999). "Evidence for three different specific saponin-detoxifying activities in Botrytis cinerea and cloning and functional analysis of a gene coding for a putative avenacinase." European Journal of Plant Pathology **105**(3): 273-283.

Quidde, T., A. Osbourn and P. Tudzynski (1998). "Detoxification of α-tomatine by Botrytis cinerea." Physiological and Molecular Plant Pathology **52**(3): 151-165.

R Development Core Team (2008). "R: A language and environment for statistical computing." R Foundation for Statistical Computing,Vienna, Austria. ISBN 3-900051-07-0.

Romanazzi, G. and S. Droby (2016). Control Strategies for Postharvest Grey Mould on Fruit Crops. Botrytis–the Fungus, the Pathogen and its Management in Agricultural Systems, Springer**:** 217-228.

Romani, L. (2004). "Immunity to fungal infections." Nature reviews. Immunology **4**(1): 1.

Rosenthal, J. P. and R. Dirzo (1997). "Effects of life history, domestication and agronomic selection on plant defence against insects: evidence from maizes and wild relatives." Evolutionary Ecology **11**(3): 337-355.

Rossi, F. R., A. Gárriz, M. Marina, F. M. Romero, M. E. Gonzalez, I. G. Collado and F. L. Pieckenstain (2011). "The sesquiterpene botrydial produced by Botrytis cinerea induces the hypersensitive response on plant tissues and its action is modulated by salicylic acid and jasmonic acid signaling." Molecular plant-microbe interactions **24**(8): 888-896.

Rowe, H. C. and D. J. Kliebenstein (2007). "Elevated genetic variation within virulence-associated Botrytis cinerea polygalacturonase loci." Molecular Plant-Microbe Interactions **20**(9): 1126-1137.

Rowe, H. C. and D. J. Kliebenstein (2008). "Complex genetics control natural variation in Arabidopsis thaliana resistance to Botrytis cinerea." Genetics **180**(4): 2237-2250.

Rowe, H. C. and D. J. Kliebenstein (2008). "Complex genetics control natural variation in Arabidopsis thaliana resistance to Botrytis cinerea." Genetics **180**(4): 2237-2250.

Samuel, S., T. Veloukas, A. Papavasileiou and G. S. Karaoglanidis (2012). "Differences in frequency of transposable elements presence in Botrytis cinerea populations from several hosts in Greece." Plant disease **96**(9): 1286-1290.

Sauerbrunn, N. and N. L. Schlaich (2004). "PCC1: a merging point for pathogen defence and circadian signalling in Arabidopsis." Planta **218**(4): 552-561.

Scala, A., L. Pazzagli, C. Comparini, A. Santini, S. Tegli and G. Cappugi (2004). "Cerato-platanin, an early-produced protein by Ceratocystis fimbriata f. sp. platani, elicits phytoalexin synthesis in host and non-host plants." Journal of Plant Pathology: 27-33.

Schumacher, J., J.-M. Pradier, A. Simon, S. Traeger, J. Moraga, I. G. Collado, M. Viaud and B. Tudzynski (2012). "Natural variation in the VELVET gene bcvel1 affects virulence and light-dependent differentiation in Botrytis cinerea." PLoS One **7**(10): e47840.

Shen, X., M. Alam, F. Fikse and L. Rönnegård (2013). "A novel generalized ridge regression method for quantitative genetics." Genetics **193**(4): 1255-1268.

Siewers, V., M. Viaud, D. Jimenez-Teja, I. G. Collado, C. S. Gronover, J.-M. Pradier, B. Tudzynsk and P. Tudzynski (2005). "Functional analysis of the cytochrome P450 monooxygenase gene bcbot1 of Botrytis cinerea indicates that botrydial is a strain-specific virulence factor." Molecular plant-microbe interactions **18**(6): 602-612.

Smale, M. (1996). "Understanding global trends in the use of wheat diversity and international flows of wheat genetic resources."

Song, H., H. Park, Y.-S. Kim, K. D. Kim, H.-K. Lee, D.-H. Cho, J.-W. Yang and D. Y. Hur (2011). "L-kynurenine-induced apoptosis in human NK cells is mediated by reactive oxygen species." International immunopharmacology **11**(8): 932-938.

Staats, M. and J. A. van Kan (2012). "Genome update of Botrytis cinerea strains B05. 10 and T4." Eukaryotic cell **11**(11): 1413-1414.

Stefanato, F. L., E. Abou‐Mansour, A. Buchala, M. Kretschmer, A. Mosbach, M. Hahn, C. G. Bochet, J. P. Métraux and H. j. Schoonbeek (2009). "The ABC transporter BcatrB from Botrytis cinerea exports camalexin and is a virulence factor on Arabidopsis thaliana." The Plant Journal **58**(3): 499-510.

Stukenbrock, E. H. and B. A. McDonald (2008). "The origins of plant pathogens in agro-ecosystems." Annu. Rev. Phytopathol. **46**: 75-100.

Tanksley, S. D. (2004). "The genetic, developmental, and molecular bases of fruit size and shape variation in tomato." The plant cell **16**(suppl 1): S181-S189.

Tanksley, S. D. and S. R. McCouch (1997). "Seed banks and molecular maps: unlocking genetic potential from the wild." Science **277**(5329): 1063-1066.

ten Have, A., W. Mulder, J. Visser and J. A. van Kan (1998). "The endopolygalacturonase gene Bcpg1 is required for full virulence of Botrytis cinerea." Molecular Plant-Microbe Interactions **11**(10): 1009-1016.

Ten Have, A., R. van Berloo, P. Lindhout and J. A. van Kan (2007). "Partial stem and leaf resistance against the fungal pathogen Botrytis cinerea in wild relatives of tomato." European journal of plant pathology **117**(2): 153-166.

Tiffin, P. and D. A. Moeller (2006). "Molecular evolution of plant immune system genes." Trends in genetics **22**(12): 662-670.

Upadhyaya, N. M., D. P. Garnica, H. Karaoglu, J. Sperschneider, A. Nemri, B. Xu, R. Mago, C. A. Cuomo, J. P. Rathjen and R. F. Park (2014). "Comparative genomics of Australian isolates of the wheat stem rust pathogen Puccinia graminis f. sp. tritici reveals extensive polymorphism in candidate effector genes." Frontiers in plant science **5**.

Vleeshouwers, V. G. and R. P. Oliver (2014). "Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens." Molecular plant-microbe interactions **27**(3): 196-206.

Weyman, P. D., Z. Pan, Q. Feng, D. G. Gilchrist and R. M. Bostock (2006). "A circadian rhythm-regulated tomato gene is induced by arachidonic acid and Phythophthora infestans infection." Plant physiology **140**(1): 235-248.

Wicker, T., S. Oberhaensli, F. Parlange, J. P. Buchmann, M. Shatalina, S. Roffler, R. Ben-David, J. Doležel, H. Šimková and P. Schulze-Lefert (2013). "The wheat powdery mildew genome shows the unique evolution of an obligate biotroph." Nature Genetics **45**(9): 1092-1096.

Yang, Q., Y. Chen and Z. Ma (2013). "Involvement of BcVeA and BcVelB in regulating conidiation, pigmentation and virulence in Botrytis cinerea." Fungal genetics and biology **50**: 63-71.

Zhang, L., A. Khan, D. Nino-Liu and M. Foolad (2002). "A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a Lycopersicon esculentum× Lycopersicon hirsutum cross." Genome **45**(1): 133-146.

Zhang, W., J. A. Corwin, D. Copeland, J. Feusier, R. Eshbaugh, F. Chen, S. Atwell and D. J. Kliebenstein (2017). "Differential Canalization across Arabidopsis Defenses against Botrytis cinerea Genetic Variation."

Zipfel, C., S. Robatzek, L. Navarro and E. J. Oakeley (2004). "Bacterial disease resistance in Arabidopsis through flagellin perception." Nature **428**(6984): 764.