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

**Nicole E. Soltis1, Susanna Atwell1, Gongjun Shi1,2, Rachel Fordyce1, Raoni Gwinner1,3, Dihan Gao1, Aysha Shafi1, Daniel J. Kliebenstein1,4**

1Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA, 95616, USA

2Gongjun current address

3Raoni current address

4DynaMo Center of Excellence, University of Copenhagen, Thorvaldsensvej 40, DK-1871, Frederiksberg C, Denmark

**\*Correspondence:** Daniel J. Kliebenstein, Department of Plant Sciences, University of California, Davis, One Shields Ave, Davis, CA, 95616, USA.

Kliebenstein@ucdavis.edu

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

**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. Resistance was slightly elevated in the wild germplasm, but interestingly there was no evidence of a bottleneck in these accessions, with wild and domesticated tomatoes showing a similar range of resistance. To complement this, we conducted genome-wide association (GWA) in *B. cinerea* that found a highly quantitative genetic 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 (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, but 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 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 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). 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 (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 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 whether the specific isolate is sensitive or resistant to camalexin (Kliebenstein, Rowe et al. 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 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 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 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, 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 and Baille 1996, Elad, Williamson et al. 2007, Fillinger and Elad 2015). Individual isolates of *B. cinerea* display the same broad host range as the generalist species (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). 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, 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). 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, Kracher et al. 2013, Wicker, Oberhaensli et al. 2013), 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, Desjardins, Cohen et al. 2016, Power, Parkhill et al. 2017). 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, 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 (Finkers, van Heusden et al. 2007, Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016). Tomato is a model system for study of the impact of domestication upon plant physiology and resistance (Tanksley 2004, Bai and Lindhout 2007, Panthee and Chen 2010, Bergougnoux 2014). This includes evidence that tomato domestication has altered the circadian clock phase (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 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 for interrogating 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 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 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 2010; Failmezger 2010} in the R statistical environment {R Development Core Team and Team 2009}, 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 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, 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 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, 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 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, Spooner et al. 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, Plotnikova et al. 2003, Denby, Kumar et al. 2004, Kliebenstein, Rowe et al. 2005, Ferrari, Galletti et al. 2007, Rowe and Kliebenstein 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 (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 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 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, 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 (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. 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, Alam et al. 2013, Corwin, Copeland et al. 2016, Corwin, Subedy 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% 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 {Kover 2002; Rowe 2008; Corwin 2016; Glazebrook 2005;

Goss 2006; Barrett 2009; Nomura 2005}. Previous studies on tomato resistance to *B. cinerea* have found a quantitative genetic architecture that varies between domesticated and wild tomato 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 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 such as Southern corn leaf blight, and pests of cotton and soybean (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*. This is in contrast to previous studies that explicitly show that there is 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 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 (Keen 1992, De Feyter, Yang et al. 1993, Abramovitch and Martin 2004, Boyd, Ridout et al. 2013, Vleeshouwers and Oliver 2014) 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, Bonazza et al. 2014, Pazzagli, Seidl-Seiboth et al. 2014). Fungal cerato-platanins have been linked to induction of systemic acquired resistance and defense compound biosynthesis in plants (Scala, Pazzagli et al. 2004, Frías, Brito et al. 2013). Chitin synthase produces a common fungal pathogen-associated molecular pattern (PAMP), and was an overrepresented function in genes linked to 10 of our 12 tomato genotypes (Table S1). However, 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, 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, Weldegergis et al. 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, Park et al. 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, Breitel et al. 2016), 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.

**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.

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.

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.

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 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, 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.

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.

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.

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.

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

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.

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.

Lannou, C. (2012). "Variation and selection of quantitative traits in plant pathogens." Annual Review of Phytopathology **50**: 319-338.

Li, R., B. T. Weldegergis, J. Li, C. Jung, J. Qu, Y. Sun, H. Qian, C. Tee, J. J. van Loon and M. Dicke (2014). "Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance." The Plant Cell **26**(12): 4991-5008.

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.

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.

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.

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.

Polturak, G., D. Breitel, N. Grossman, A. Sarrion‐Perdigones, E. Weithorn, M. Pliner, D. Orzaez, A. Granell, I. Rogachev and A. Aharoni (2016). "Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants." New Phytologist **210**(1): 269-283.

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.

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.

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.

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.

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.