**Natural variation for *Botrytis cinerea* virulence and susceptibility of domesticated and wild tomato**

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**Abstract**

**Introduction**

Plant disease involves complex interactions between the molecular pathways of the host and pathogen. The resulting disease can be viewed as the sum of pathogen virulence/sensitivity and host susceptibility/resistance. Focusing on plant-pathogen interactions, pathogens are often classified based on their host range. Specialist pathogens are virulent only on a narrow range of hosts. Suitable hosts may be limited to a single species or genus, leading to co-evolution between host and pathogen. This facilitates selection by pathogen virulence genes for altered host resistance genes, and vice versa. Most known genes for plant resistance to specialist pathogens confer qualitative resistance through plant innate immunity {Dodds 2010; Pieterse 2012}. This frequently involves large-effect, qualitative R-gene mediated resistance, in which alleles at a single plant resistance locus (R-gene) and a single pathogen avirulence locus determine susceptibility, based on recognition of the pathogen gene by the plant R gene {Dodds 2010; Dangl 2001; Jones 2006}. For example, pattern recognition receptors in plants induce defense pathways following sensing of a conserved pathogen signal, such as cell-wall polymers or flagellin. The reciprocal selective pressures present in interactions between hosts and specialist pathogens makes evolution of these specific gene-for-gene interactions relatively common.

In contrast to specialist pathogens, generalist pathogens cause disease in diverse hosts across taxa. They may be less sensitive to variation in host susceptibility/resistance gene evolution because of their ability to shift niche by moving from host to host. Thus, generalist pathogens can evade detrimental shifts in specific hosts, making evolution of gene-for-gene interactions or large effect qualitative resistance rare. As such, most naturally variable plant genes for resistance to generalist pathogens are quantitative in their effect, rather than qualitative. There are no known naturally variable resistance loci with large effects on qualitative plant defense against generalist pathogens such as *Botrytis cinerea*  {Rowe 2008; Corwin 2016; Glazebrook 2005}. Modern genomic approaches are rapidly identifying a broad array of loci that control quantitative resistance to generalist pathogens in plants. These include genes involved in the formation of defenses like secondary metabolites, cell walls and defense proteins as well as genes involved in the signaling cascades that link the perception of the pathogen to the defense output (Ferrari, Galletti et al. 2007). The effect of these quantitative plant resistance loci is highly dependent upon genetics within the infecting pathogen. However, very little is known about the genetic variation of virulence loci within generalist pathogens. 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. Thus, to truly understand quantitative host-pathogen interactions, we need to work with genetic variation in both the host and pathogen.

Plant domestication has large effects on plant genetic variation, including variation controlling pathogen resistance. In response to specialist pathogens, domesticated plant varieties are typically more sensitive than their wild relatives. Domestication poses a strong genetic bottleneck, reducing diversity genome-wide. In particular, domesticated host susceptibility may increase due to bottlenecks at pathogen resistance loci. This loss of resistance is assumed to extend to all domesticated varieties, particularly if cultivated plants experience reduced selective pressures from pathogens. In contrast, domesticated plants can experience increased selective pressures from some pathogens. These evolutionary patterns are assumed to hold for generalist pathogens and their domesticated hosts as well. However, we have less information about how domestication of hosts affects disease due to generalist pathogens. It is possible that generalist pathogens are relatively insensitive to domestication and these genetic bottlenecks.

*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, Copeland et al. 2016) in contrast to pathogens like *Fusarium oxysporum* where the species can infect a number of hosts, but each isolate is highly host specific (Katan 1999). Even though *B. cinerea* isolates have broad host ranges, individual isolates display significant variation in virulence phenotypes. Genetic variation in the production of the phytotoxins, botrydial and botcinic acid, differentially controls virulence on host plants including tomato (Siewers, Viaud et al. 2005, Dalmais, Schumacher et al. 2011). Additionally, *B. cinerea* has genetic variation in virulence genes which control degradation of different plant cell walls. This genetic variation appears to contribute to quantitative differences in virulence (ten Have, Mulder et al. 1998). Further, natural variation at three *B. cinerea* polygalacturonase loci contributes to virulence on plant hosts, likely through diversifying selection and some host specialization of individual loci (Rowe and Kliebenstein 2007). More recently, natural variation in VELVET, a gene involved in development and secondary metabolism, was found to be necessary for oxalic acid production. Variation in VELVET led to quantitative variation in virulence on multiple host plants (Schumacher, Pradier et al. 2012). Isolates of *B. cinerea* are also highly variable genome-wide; at XX it is more variable than previously studied pathogens, and on par with XXXX (CITATION). As such, *B. cinerea* has the potential for identifying natural genetic variation controlling quantitative virulence.

On the plant side, plant resistance to *B. cinerea* is dominated by quantitative and highly polygenic resistance, with no evidence for qualitative resistance (Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016)*.* A model pathosystem for studying quantitative interactions is the tomato-*B. cinerea* system, where the pathogen causes crop loss due to both pre- and post-harvest infection. Resistance to *B. cinerea* is a quantitative trait in tomato, with identified tomato QTLs explaining up to 15% of phenotypic variation in a stem bioassay (Finkers, van Heusden et al. 2007). Tomato is a model system for study of the impact of domestication upon plant physiology and resistance. This includes evidence that tomato domestication has altered the circadian clock phase {Muller 2016}, which can modulate resistance to *B. cinerea* {Sauerbrunn 2003; Bhardwaj 2011; Weyman 2006}. Further, *B. cinerea* infection can influence the amplitude of circadian oscillations in clock gene expression (Windram, Madhou et al. 2012), and both the plant and pathogen clocks impact *B. cinerea* virulence (Hevia, Canessa et al. 2015). This suggests that host plant domestication can alter traits known to influence *B. cinerea* resistance from other systems. Thus, the tomato-*B. cinerea* pathosystem allows us to directly test how genetic variation in a generalist pathogen may be influenced by domestication in a crop plant.

In this study, we conducted genome-wide association (GWA) in the pathogen to see how it broadly responds to host phenotypic variation, and more specifically to domestication. 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, controlled by genetics in both the plant and the pathogen (Rowe and Kliebenstein 2008). In our analysis of lesion images at 72 hours post inoculation, both host and pathogen genotypes contribute to virulence. Our findings suggest that individual isolates are generalists across tomato genotypes and across domestication in *Solanum.* A subset of single isolates, however, are sensitive to tomato domestication. We do not find evidence for host specialization; *B. cinerea* isolates collected from tomato tissues are not within the most-virulent isolates on tomato. 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.

**Methods**

**Tomato genetic resources**

We obtained seeds for 12 selected tomato genotypes in consultation with Dr. Roger Chetelat at the UC Davis TGRC. These include a diverse sample of 6 genotypes of domesticated tomato’s closest wild relative (*S. pimpinellifolium*) from throughout its native range (Peru, Ecuador) as well as 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. Plants were grown 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. Plants were pruned and staked upright, and fruits were collected as they matured.

Fruits were stored at 4°C in dry paper bags until seed cleaning. Seeds and locule contents were incubated at 24°C in 1% protease solution (Rapidase C80 Max) for 2h, then rinsed in dI H2O and air-dried. Seeds were then stored in a cool, dry, dark location until further plantings.

We bleach-sterilized all seeds prior to germinating on germination paper in growth chambers. At 7 days we transferred seedlings to soil (SunGro) and grew all plants in growth chambers in 20°C, short-day (10h photoperiod) conditions with 180-190 uM light intensity and 60% RH. The flat was covered with a humidity dome during germination. We bottom-watered with dI H2O 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). Plants were used for detached leaf assays 6 weeks after seedlings were transferred to soil.

**Botrytis genetic resources**

We sourced the *Botrytis cinerea* collection from single-spore isolates from fruit and vegetable tissues as described by Atwell {Atwell 2015}. DNA was extracted from *B. cinerea* hyphal cells and sequenced by Illumina GAIIx or HiSeq as described by S. Atwell {Atwell 2015}. Sequencing data was cleaned, aligned, and variant calls were made as previously described {Atwell 2015}. For the 91 isolates used in this study, we utilized a total of 272,672 SNPs with MAF 0.20 or greater, and less than 10% missing values (SNP calls in at least 82/ 91 isolates).

**Botrytis growth**

Botrytis isolates were maintained as conidial suspensions in 30% glycerol for long term storage at -80°C. For regrowth, spore solutions were diluted to 10% in 50% filter-sterilized grape juice, then inoculated onto 39g/L potato dextrose agar (PDA) media. Isolates were grown at 25°C in 12h light, and propagated every 2 weeks.

**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 91 Botrytis isolates. We used a randomized complete block design for a total of 6 replicates across 2 experiments. Leaflets were placed on 1% phytoagar in seed flats, with humidity domes on top.For each plant genotype, leaflets from each of 10 plants were placed onto agar in blocks. Leaves were selected by a random sample of 5 leaves per plant, and 2 leaflet pairs per leaf.

Spores were collected from mature (1-2 week old) Botrytis cultures, and diluted to 10 spores/ uL in 50% filter-sterilized grape juice. 4ul droplets of spore suspensions were inoculated onto detached leaves at room temperature with 24h light. Control leaves were mock-inoculated with 4uL of grape juice without spores. Lesion development was measured using digital photos of all leaflets at 24, 48, and 72 hours post inoculation in combination with downstream automated image analysis.

**Automated Image Analysis**

We measured lesion areas using the EBImage and CRImage packages (Pau et al., 2010; Failmezger et al., 2010) in the R statistical environment (R Development Core Team and Team, 2009). 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 by F-test the 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 species), experiment, and block (nested within experiment) on lesion area. We next included terms for the interactions of plant domestication with isolate, plant genotype with isolate, and experiment with isolate, plant domestication, or plant genotype as fixed effects. Adding terms for individual plant, leaf, and leaflet position did not significantly improve the full model, so we 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. Next we calculated the least-squared means of lesion size within each tomato genotype. For the within-genotype model we included the fixed effect of isolate, and the random effects of experiment, the isolate by experiment interaction, and leaflet pair (nested within leaf, nested within individual plant). We then used these means as the phenotype input to our custom bigRR script for GWA. SNP data included 272,672 loci at MAF 0.20 or greater as described above. We imputed missing SNPs in bigRR. Because bigRR provides an estimated effect size, but not a p-value, we performed permutation analyses to determine effect significance. We permuted the phenotypes 1000x and re-ran bigRR, to establish 95%, 99%, and 99.9% thresholds for significance. We performed SNP annotation using SNPdat (Doran and Creevey 2013) with gtf construction from the T4 gene models for genomic DNA ([http://www.broadinstitute.org](http://www.broadinstitute.org/), (Staats and van Kan 2012)). We used the program InterProScan within BLAST2GO for functional annotation of the gene models (http://www.blast2go.com).

**Results**

**Experimental Design**

To directly measure the impact of tomato domestication on quantitative resistance, we infected with a collection of 91 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 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 genotypes that were the founders of mapping populations, and found a wide range of pathogen susceptibility levels both within and between tomato species. However, it is still unknown how domesticated and closely related wild tomatoes compare for *B. cinerea* resistance using multiple plant genotypes. We selected *S. pimpinellifolium*, the closest wild relative of *S. lycopersicum*, to directly study the selection associated with the impact of domestication (Peralta, Spooner et al. 2008). We selected tomato genotypes including 6 domesticated *Solanum lycopersicum* cultivars and 6 wild *S. pimpinellifolium* genotypes. We isolated 91 *B. cinerea* genotypes from various eudicot plant hosts, including tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2, Supersteak). We infected all 91 *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 the developing lesion 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-*Botrytis* interaction has been used successfully in a number of molecular and quantitative genetic studies {Ferrari 2003; Denby 2004; Kliebenstein 2005; Ferrari 2007; Rowe 2008}.

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

We wanted to know the relative contribution of genetic variation in both the plant and the pathogen to variation in the virulence/ susceptibility phenotype. Using a linear model, we asked how plant genotype, plant domestication status, and pathogen genotype (isolate) contribute to variation in lesion size. The final model explains 60% of the variance for lesion size, and shows that genetic variation within both the host plant and the pathogen have significant effects on lesion growth, but isolate explains 3.5x more of the variance than plant genotype (10.2% of total variance under isolate vs. 2.9% under plant, Table R1). Interestingly, tomato domestication significantly impacted *B. cinerea* virulence, as shown by the significant effects of tomato genetic variation between domesticated and wild species (<1% of total variance, Table R1). There was no evidence for significant interaction effects between isolate and plant genotypes 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 caused by vast number of degrees of freedom in this term (Table R1). In short, lesion size is controlled by genetics both within the host plant and the pathogen. The question remains, at what level does genetic variation in the host contribute to lesion growth? Host genotype may be the major determinant of plant susceptibility to *B. cinerea*, or host domestication status may be more relevant.

**Domestication and Lesion Size Variation**

Existing literature predominantly theorizes that crop domestication increases plant susceptibility to pathogens {Stuckenbrock 2008}(CITATIONS). In our model analysis, we identified a significant difference in the resistance of wild and domesticated tomato (p <2e-16, Table R1). This agreed with the theory that domestication decreases resistance, as the average lesion size is slightly greater on domesticated than on wild tomato genotypes (18% increase) (Table R1, Figure R2). However, this domestication effect is not the dominant source of variation as genetic variation within the domesticated and within the wild genotypes contributes 3.8 fold more variation in resistance than domestication alone (2.9% vs. 0.78% of total variance, Table R1). So while we do observe the expected increase of susceptibility in domesticated tomato, domestication does not explain the major effects of tomato genotype on lesion size variation and there is significant remaining genetic variation in *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 {Doebley 2006; Tanksley 1997; Bai 2007}. We would expect this decreased genetic variation to restrict phenotypic variation, including disease phenotypes. Interestingly in this 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 coefficient of variation (CV) of lesion size does not statistically differ between domesticated and wild tomato (F-test, F=1.39, 96 df, p=0.11)(Figure R3). Rather than reduced variation for lesion size across domesticated tomato genotypes in response to a domestication bottleneck, we observe an increased range of lesion sizes in domesticated compared to wild tomato. Overall, we see evidence for a slight domestication impact on average resistance to *B. cinerea* that depends on the host genotype, but no evidence of a phenotypic bottleneck due to domestication.

**Pathogen Specialization to Source Host**

One model of generalist pathogens suggests that isolates within generalist species may adapt to specialize on specific hosts. Alternately, isolates may also be generalists, with specialization absent or occurring only at the gene level. Our collection includes five single-pathogen isolates from *S. lycopersicum*, potentially adapted to tomato. Within our collection, there was a significant effect of genetic variation in the 91 *B. cinerea* isolates across all the plant genotypes (Table R1 and Figure R4A). To test if there is evidence for specialization to the source host, we compared the virulence of the *B. cinerea* isolates from tomato in comparison to our broader pathogen population. For *B. cinerea* genotypes isolated from tomato tissue vs. other hosts, we find no significant difference in lesion size across all hosts on domesticated tomato (t-test; t=1.10, 4.3 df, p=0.33), wild tomato (t-test; t=1.09, 4.2 df, p=0.33) or across all tomato genotypes (t-test; t=1.60, 9.7 df, p=0.14) (Figure R4E). In fact, one isolate collected from tomato tissue (KGB1) is within the 10 least-virulent isolates in this study (Figure R4E), and one is within the 10 most-virulent isolates (Triple3). This shows that there is significant genetic variation in virulence across the *B. cinerea* isolates and supports the general observation that *B. cinerea* has minimal host-specificity {Rowe 2007}(Citations).

**Pathogen Specialization to Host Variation**

Though we did not find evidence for *B. cinerea* adaptation to tomato based on isolate source host, *B. cinerea* may be adapted to individual tomato genotypes. A visual analysis of the data showed that lesion size for many isolates varies across the tomato genotypes, suggesting 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 specialization in *B. cinerea* to tomato host genotypes. However, this negative result may also be because F-tests with high degrees of freedom can be underpowered, as in the case of the isolate x plant genotype interaction term (df: 940). We took an additional approach to statistically test for an interaction between *B. cinerea* and host genotype. We split the data by isolate, and within each new dataset performed GLM ANOVA with the fixed effects of domestication, plant genotype nested within domestication, and experiment. Through this single-isolate GLM analysis, none of the isolates show a significant (p < 0.05, FDR corrected) interaction with host genotype, so we do not see evidence of sensitivity to genetic variation in tomato in these isolates.

Because some isolates showed differential sensitivity to genetic variation within tomato, we used the same approach to test if specific isolates, independent of their host, may show sensitivity to genetic variation associated with tomato. Under the single-isolate ANOVAs including the fixed effects of plant, domestication, and experiment, two isolates showed 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 saprophytic isolates (Rose), suggesting that *B. cinerea* adaptation to tomato domestication is not dependent on isolate virulence. Both of these isolates are more virulent on domesticated than on wild tomato. Further, isolate ranking by mean lesion size differs between domesticated and wild hosts (Wilcoxon signed-rank test, V=4322, p=2.586e-12) (Figure R3). These domestication-sensitive isolates may be adapted to domesticated tomato, or more broadly to domesticated plants.

**Quantitative Genetics of Pathogen Virulence on Tomato**

With some evidence for isolate-level adaptation of *B. cinerea* to tomato variation, we asked whether we could find evidence of *B. cinerea* adaptation at the genetic level to tomato. While we did not see much isolate-level specialization to tomato, there may be more specialization at the genetic level. Due to the large effect of plant genotype on resistance to *B. cinerea*, we performed GWA on each plant genotype independently. We calculated least-squared means of lesion size for each isolate from linear models within each plant genotype, including the effects of isolate, experiment, and individual plant. We used a ridge-regression approach (Shen, Alam et al. 2013) to calculate GWA of *B. cinerea* SNP variation for the 91 isolates and the lesion size phenotype. To determine significance of SNP effects, we permuted phenotypes 1000x to calculate 95, 99, and 99.9% thresholds within each plant host.

Initial GWA analysis revealed that the basis of *B. cinerea* virulence on tomato is highly polygenic, as is *A. thaliana* resistance to *B. cinerea* {Corwin 2016}. On all of the hosts, many SNPs had effect size estimates exceeding the 99% permutation threshold, ranging from 1284 to 24669 SNPs per host. For the remaining 3 domesticated hosts, at least 1400 SNPs exceeded the 95% threshold. For one host, LA1547 (wild), no SNPs were significantly associated with *B. cinerea* lesion size.

Candidate loci which are identified across multiple tomato host genotypes may tell us general strategies for *B. cinerea* virulence in tomato. We found significant overlap in *Botrytis* candidate loci identified in different plant host genotypes. For the eight host plants with SNPs > 99.9%, we looked for overlap in significant SNPs. A total of 13 SNPs were called in all eight of these hosts (Figure R5B), and 25 additional SNPs were called in at least half of the hosts. Dozens more occurred in two or more hosts. However, hundreds of additional loci were identified only from a single host genotype, indicating that the genetic basis of *Botrytis* virulence on tomato is host-dependent. This is in contrast to our phenotypic results which found weak evidence for a *Botrytis*-tomato interaction in determining virulence; the interaction between this pathogen and its host may be at the genetic, rather than the genotypic, level.

We next examined the top 50 SNPs for each plant host (Figure R6). 12 SNPs have dramatically larger effect sizes on multiple hosts but this was still only 3-7%. The rest of the SNPs had much smaller effects. Thus, the pathogen appears to rely on polygenic small effect loci to control virulence on the different tomato hosts.

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

To directly test the *Botrytis* genetics underlying the domestication lesion size phenotypes, we again calculated least-squared means of lesion size for each isolate from linear models. This time we calculated least-squared means within all domesticated hosts, within all wild hosts, and the phenotype of domestication sensitivity; the difference in lesion size for each isolate between domesticated vs. wild hosts. We conducted GWA within *B. cinerea* for each of these domestication linked phenotypes; domesticated, wild, and domestication sensitivity. Numerous SNPs exceeded the 99% threshold for domestication phenotypes as well as the phenotype of lesion size on individual plant genotypes (Figure R6). *B. cinerea* response to tomato domestication appears to be polygenic, with many loci of small effect sizes which are trait dependent. Domestication sensitivity often identified unique SNPs from domesticated or wild alone (Figure R7; Figure R8).

We annotated genes from *B. cinerea* T4 gene models within 2kb of significant SNPs. At the gene level, 43 genes were associated with domesticated, wild, and domestication sensitivity phenotypes, but 60 genes were uniquely identified by a single domestication phenotype (Figure R10)]. A total of 142 genes contained significant SNPs (>99.9%) when studied for one or more of the domestication phenotypes (Table S1). Broadly, 50 of these are enzymes, 16 are involved in cellular processes, 7 in DNA structural modification, 6 are transcription factors, 5 involved in defining mating types, 4 in redox regulation, 1 in detoxification, and 1 in pathogenesis. This suggests that most variation in *Botrytis* genetic control of virulence acts to change biochemistry in the pathogen. Notably, only a single gene predicted to be associated with pathogenesis was identified, containing a CFEM domain.

When examining the top 50 SNPs for lesion size on each individual plant genotype, we identified 93 genes within only a single plant host, and 12 genes within 8 of the hosts. The list of top 50 SNPs per host covers a total of 153 genes and a few regions with no gene model identified. Among these genes, 45 are enzymes, 16 are involved in cellular processes, 3 in DNA structural modification, 10 in transcriptional regulation (6 TFs), 6 in defining mating types, and 3 in redox regulation (Table Sx).

**DISCUSSION**

Summary paragraph

**Domestication and altered pathogen virulence genetics**

These results provide evidence of a mild host domestication effect on resistance to the generalist pathogen, *Botrytis cinerea.* However, domestication status alone is a poor predictor of host response to infection by *B. cinerea*. This suggests that while plant domestication does affect plant-pathogen interactions, it is not the primary evolutionary force in defining these interactions. 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. Host domestication only significantly affected three out of the 91 isolates we studied. So while host domestication consistently reduces resistance to this generalist pathogen, this may be driven by a domestication-sensitive subset of *B. cinerea* genotypes. Given that the effect of host domestication varies by *B. cinerea* genotype, this supports the approach of studying natural variation within *B. cinerea* to understand the factors contributing to *B. cinerea* virulence. 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. This assumption is supported in studies of specialist pathogens [GIVE EXAMPLES]. Surprisingly, we did not find evidence for a domestication bottleneck in resistance to *B. cinerea*. This contradicts our expectation of a genome-wide loss of variation through domestication. In fact, the increased phenotypic diversity for resistance suggests increased genotypic diversity. This could be due to recombination between domesticated lines, as new combinations of alleles are mixed together.

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 *Botrytis* 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 effect size of individual SNPs is very small (on the scale of 0.01 mm2), and many SNPs, approximately 1200 – 25,000 on each plant genotype, are associated with *B. cinerea* virulence. This is in sharp contrast to the few genes involved in quantitative virulence of specialist pathogens. Further studies can explore the number and effect sizes of SNPs contributing to virulence of *B. cinerea* on other hosts.

Our results indicate some particular challenges for breeding durable resistance to generalist pathogens. The highly quantitative nature of *B. cinerea* virulence, and the variation between isolates, suggests that we cannot clone or introgress single genes to breed durable resistance against this pathogen. In contrast, we will likely need to work on breeding resistance through targeting entire pathways or mechanisms. In order to breed resistance to *Botrytis cinerea* or other generalist pathogens, it is likely necessary to work with a genetically variable population. This study indicates that responses to host domestication, host genotype, and virulence genetics vary with pathogen genotype. Breeding resistance to a single pathogen genotype is unlikely to translate to durable resistance against B. cinerea as a species. The mild domestication effect on resistance suggests that, at least for tomato, we need not introgress genes from wild relatives to breed resistance to *B. cinerea*. The genetic diversity within domesticated tomato should be sufficient to identify alleles for resistance.

**Molecular mechanisms and polygenic virulence**

The genetics of *B. cinerea* virulence do not conform to our expectations based on the genetics of virulence in specialist pathogen studies. The mechanisms of quantitative virulence identified in this study are in contrast to previously-described qualitative virulence loci. Major mechanisms we identified include enzymatic pathways and [XX more here]. The classic qualitative resistance pathways of pathogen sensing (receptors) and signaling (immune response pathways) are not the major contributors to quantitative resistance. We did not identify any MAMPs or PAMPs as major loci contributing to virulence across tomato varieties, nor any chitins, glycans, or glycolipids which are often recognized by plant receptors for qualitative resistance. Nor did we identify any mannans as top contributors to *B. cinerea* virulence [JAC + Klieb citation]. Further, our identitifed loci did not include any known virulence loci, such as NEPs, or PGs. We did identify some unknown glycosyl transferases. These may function in cell wall degradation, phytoalexin degradation, or other functions.

**Conclusion**

**FIGURES**

Table R1. Results of ANOVA from GLM of *Botrytis cinerea* lesion area. Isolate is 91 *B. cinerea* genotypes, Domestication is *S. pimpinellifolium* or *S. lycopersicum*, Plant is 12 tomato genotypes nested within Domestication, Experiment is 2 replicate experiments, Block is 3 replicates nested within Experiment. Slash / indicates nesting, colon : indicates interactions between factors.

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). Digital masking of leaf and lesion (B) is followed by automated measurement of area for each lesion.

Figure R2. Relative susceptibility of tomato genotypes to *B. cinerea* infection. Violin plots are of lesion size due to *Botrytis cinerea* growth on each tomato host genotype. Individual points are mean lesion size for each isolate-host pair.

Figure R3. *B. cinerea* virulence responds to host domestication. The violin plots include each *B. cinerea* lesion on the host species. The interaction plot traces the average lesion size of a single *B. cinerea* isolate across the host species.

Figure R4. *Botrytis cinerea* virulence varies due to isolate-host interactions. Interaction plot of lesion size due to individual *B. cinerea* isolates on tomato host genotypes. The x-axis includes each tomato host genotype. Each line traces the average lesion size of a single *B. cinerea* isolate across hosts. A is a plot of all isolates, B-F highlight a subset of isolates. B is B05.10, C is the 10 most highly-virulent isolates, D is the 10 most saprophytic (low-virulence) isolates, E is 5 isolates collected from tomato tissue, F is 2 domestication-sensitive isolates.

Figure R5. *B. cinerea* lesion size is a polygenic trait on tomato. A: Manhattan plot of *B. cinerea* lesion size GWA for a single accession, LA2706. B: Overlap in lesion size SNPs > 99% threshold across multiple host plant phenotypes. Chromosomes are differentiated by shading. Frequency is number of phenotypes in which the SNP exceeds the threshold. Vertical dotted lines indicate overlap between relatively large-effect SNPs for LA2706 and significance across the majority (≥6) of tomato genotypes tested.

Figure R6. Top 50 SNPs for lesion size phenotype on each host plant. Points are color coded by plant host.

Figure R7. Overlap in lesion size SNPs > 99% across individual-host phenotypes and domestication phenotypes. A: Count of SNPS > 99% in common across individual plant hosts. B: Count of SNPs >99% across phenotype categories. Pale green is X, lilac is X, turquoise is X.

Figure R8. A: Top 50 SNPs for lesion size for each domestication phenotype. Domestication sensitivity is (domesticated – wild / domesticated). B: Venn diagram of SNPs identified >99.9% for each domestication phenotype. C: Venn diagram of genes with a significant SNP identified >99.9% for each domestication phenotype.

**References**

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.

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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.

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.

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.

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

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.

Windram, O., P. Madhou, S. McHattie, C. Hill, R. Hickman, E. Cooke, D. J. Jenkins, C. A. Penfold, L. Baxter and E. Breeze (2012). "Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis." The Plant Cell **24**(9): 3530-3557.