**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 cumulative sum of pathogen virulence/sensitivity and host susceptibility/resistance. Pathogens can be classified based on their host range. Specialist pathogens exhibit virulence 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 allows evolutionary interactions such that changes in pathogen virulence genes will select for altered host susceptibility genes and vice versa. Most known genes for plant resistance to specialist pathogens confer qualitative resistance through plant innate immunity. 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. 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 infect 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 niche. This allows generalist pathogens to evade non-favorable shifts in specific hosts and makes the evolution of gene-for-gene or large effect qualitative resistance difficult.

In plants, most naturally variable genes for resistance to generalist pathogens are quantitative in their effect, rather than qualitative. There are no known naturally variable large-effect resistance loci for plant defense against generalist pathogens such as *Botrytis cinerea* (Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016). 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 resistance loci is highly dependent upon the specific isolate of the generalist pathogen. In contrast, very little is known about the genetic variation of virulence loci within generalist pathogens. There are no known reported naturally variable large-effect virulence loci in generalist pathogens, suggesting that quantitative genetic variation in these pathogens modulates virulence. Thus, to truly understand quantitative host-pathogen interactions, we need to work with genetic variation in both the host and pathogen.

Plant domestication has altered the evolution of host-pathogen interactions. For specialist pathogens, domesticated 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 selective pressures from pathogens are reduced under cultivation. In contrast, domesticated plants may experience increased selective pressures from some pathogens. These 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 generalist pathogens. It is possible that generalist pathogens are relatively insensitive to domestication and these genetic bottlenecks.

**Selected pathogen-host system**

*Botrytis cinerea* provides a useful model generalist pathogen to study its quantitative interactions with plant hosts, and to contrast how the underlying evolutionary processes may differ from evolution in 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 crops {Nicot 1996; Elad 2007; Fillinger 2015}. Individual isolates of *B. cinerea* display the same 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 this is because each isolate is highly host specific (Katan 1999). Even though individual *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 control virulence on various host plants including tomato (Siewers, Viaud et al. 2005, Dalmais, Schumacher et al. 2011). *B. cinerea* also has genetic variation in virulence genes which control degradation of different plant cell walls, which appears to lead to quantitative differences in virulence (ten Have, Mulder et al. 1998). More recently, natural variation in VELVET, a development and secondary metabolism gene, was shown to be necessary for oxalic acid production. This led to quantitative variation in virulence on multiple host plants (Schumacher, Pradier et al. 2012). Genome-wide variation in *Botrytis* is also high; 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.

Quantitative and highly polygenic resistance is the dominant form of resistance to *B. cinerea* in plants with no evidence for qualitative resistance to *B. cinerea* (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 QTLs explaining up to 15% of phenotypic variation in a stem bioassay (Finkers, van Heusden et al. 2007). Tomato is also 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}. This suggests that domestication can alter traits known to influence *B. cinerea* resistance in 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 a detached leaf assay. 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 genotype 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.

[ADD: GWAS results. highly quantitative trait. Number of loci/ genes per phenotype. And: GO terms]

**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**

[Selection of genotypes / population collection]

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 XXXX SNPs with MAF XXX.

**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 effects of isolate, plant domestication, plant genotype (nested within domestication), experiment, block, and the interaction effects of plant with isolate, experiment with isolate, and experiment with plant. Next we calculated the least-squared means of lesion size within each tomato genotype. 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 XXX loci at MAF XXX as described above. (get SNP details from Suzi). Because bigRR provides an estimated effect size, but not a p-value, we perform permutation analyses to determine effect significance. We permute the phenotypes 1000x and re-run bigRR, to establish 95%, 99%, and 99.9% thresholds for significance. SNP annotation was performed using SNPdat {Doran 2013} with gtf construction from the T4 gene models for genomic DNA ([http://www.broadinstitute.org](http://www.broadinstitute.org/), {Staats 2012}.

**Results**

**Experimental Design**

To directly measure the impact of tomato domestication and genetic variation on quantitative resistance, we infected with a collection of 91 diverse *B. cinerea* isolates on 6 wild and 6 domesticated tomato genotypes. Previous studies have examined the contrast in *B. cinerea* resistance between wild and domesticated tomato using distantly related wild species {Nicot 2002; Ten Have 2007; Egashira 2000; Guimaraes 2004; Finkers 2008}. These single-isolate studies found a wide range of pathogen susceptibility levels both within and between tomato species, though none of the studies directly compared wild versus domesticated genotypes. We selected *S. pimpinellifolium*, the closest wild relative of *S. lycopersicum*, to directly study the selection associated with the impact of domestication {Peralta 2008}. We selected tomato genotypes including 6 domesticated *Solanum lycopersicum* cultivars and 6 wild *S. pimpinellifolium* genotypes. The 91 *B. cinerea* genotypes used were isolated 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 plant genotype 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 successfully utilized in a number of molecular and quantitative genetic studies {Rowe 2008; Denby 2004; Ferrari 2003}.

Using the individual lesion measurements, we performed statistical analysis using a linear model. Within the initial model, we tested effects of isolate genotype, plant species (domesticated or wild), plant genotype (which is nested within species), experiment, block (nested within experiment), as fixed effects. We also included terms for the interactions of plant species with isolate, plant genotype with isolate, and experiment with isolate, plant species, or plant genotype as fixed effects. Terms for individual plant, leaf, and leaflet position did not significantly improve the full model, so we omitted them from this analysis. The final model shows that genetic variation within both the host plant species and the pathogen significantly affect lesion growth (Table R1). Interestingly, the differences due to tomato domestication also significantly impacted lesion formation, as shown by the significant effects of tomato genetic variation between domesticated and wild species. There was no evidence for significant interaction effects between isolate and plant genotypes but this term contributed the largest proportion of the variance in lesion size (Table R1). This lack of significance may have been caused by the vast number of degrees of freedom in this term (Table R1). CONCLUSION

**Domestication and Lesion Area**

Comparing the domesticated and wild tomato genotypes showed that lesion size is slightly greater on average (18% increase) on domesticated tomato compared to wild tomato (p <2e-16, Table R1) (Figure R2). This agrees with existing literature that has proposed that domestication increases susceptibility to pathogens {Stuckenbrock 2008}(CITATIONS). While domestication is significant, it contributes 3.8 fold less variance than the effect of genetic variation between the individual plant genotypes (Table R1). Another common observation is that domestication decreases genetic variation in the domesticated germplasm in comparison to the wild germplasm due to selection bottlenecks during domestication including for tomato {Doebley 2006; Tanksley 1997; Bai 2007} (CITATIONS). Interestingly in this population, the domesticated tomato genotypes had a wider range of average lesion size than wild genotypes with the 90 percentile range (95th percentile to 5th percentile) being 2.03 cm2 lesion size variation on domesticated tomato versus 1.76 cm2 variation in the wild tomato genotypes. Additionally, the ordering of isolates by coefficient of variation (CV) of lesion size does not statistically differ between domesticated and wild hosts (Wilcoxon signed-rank test, V=2275, p=0.7163), indicating a lack of evidence for a domestication effect on lesion size variance (Figure R3). A domestication bottleneck would lead to reduced variation for lesion size across domesticated tomato genotypes; instead 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 *Botrytis cinerea* that depends on the host genotype, but there is no evidence of a phenotypic bottleneck.

**Pathogen Variation and host specialization**

In addition to a significant effect of plant host, there was a significant effect of genetic variation in the 91 *B. cinerea* isolates across all the plant genotypes (Table R1 and Figure R4A). We did not detect a significant interaction between *B. cinerea* genotype and tomato host genotype, which may indicate a lack of host specialization in *B. cinerea* (Table R1). To test if there is any evidence for host specialization, we compared the virulence of the *B. cinerea* isolates from tomato against the entire collection of isolates. For *B. cinerea* genotypes isolated from tomato tissue vs. other hosts, there is no significant difference in lesion size across all hosts on domesticated (t-test; t=-1.10, 4.3 df, p=0.330), wild (t-test; t=-1.09, 4.2 df, p=0.332) or across all tomato genotypes (t-test; t=-1.5972, 9.65 df, p=0.142) (Figure R4F). In fact, one isolate collected from tomato tissue (KGB1) is within the 10 least-virulent isolates (Figure R4F). This shows that there is significant genetic variation in virulence across the *B. cinerea* isolates and supports the general observation that there is minimal host-specificity (Citations).

**Pathogen Variation and the Interaction with the Host Domestication**

A visual analysis of the data suggested that lesion size for many isolates varies across the host genotypes, suggesting an interaction between the genomes of *B. cinerea* and tomato (Figure R4). However, when using the full model, there was no significant interaction between isolate genotype and either domestication status or individual host genotype but there was a large fraction of variance within each term (Table R1). This is likely due to the many degrees of freedom in calculating this interaction effect. As such, we took alternate approaches to examine the interaction between domestication and isolate in determining lesion size. We performed ANOVA for each isolate examining the fixed effects of plant and domestication, and the random effect of experiment. Following FDR correction for multiple testing, three isolates showed a significant effect of domestication on lesion size (Figure R4F). These included one of the highly virulent isolates, an intermediate isolate, and one of the saprophytic isolates, suggesting that the domestication effect is not dependent on isolate virulence.

Isolate ranking by mean lesion size differs between domesticated and wild hosts (Wilcoxon signed-rank test, V=4322, p=2.586e-12) (Figure R3).

**Pathogen Variation and the Interaction with the individual host variation**

Tomato genotype within each species did not have a significant interaction effect with isolate genotype (Table R1). The F-test could identify significant effects of isolate and species but not the interaction between them, because F-tests with high numbers of degrees of freedom can be significantly 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 dataset 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, a subset of isolates show a significant (p < 0.05) interaction with host genotype (Figure R4E).

**Quantitative genetics of pathogen virulence**

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 2013} to calculate GWA of *B. cinerea* SNP variation for the 92 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. On three of the domesticated hosts and five of the wild hosts, many SNPs had effect size estimates >99.9%, ranging from 140 to 324 SNPs per host. For the remaining 3 domesticated hosts, at least 150 SNPs exceeded the 95% threshold. For one host, LA1547 (wild), no SNPs were significantly associated with *B. cinerea* lesion size.

For the eight host plants with SNPs > 99.9%, we looked for overlap in significant SNPs. A total of 4 SNPs were called in all eight of these hosts (Figure R5), and 4 additional SNPs were called in at least half of the hosts. Dozens more occurred in two or more hosts.

We also examined the top 50 SNPs for each plant host (Figure R6). 6 SNPs have dramatically larger effect sizes on multiple hosts but this was still only (effect size). 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.

We next directly examined the phenotype of domestication effects on lesion size. For this, we again calculated least-squared means of lesion size for each isolate from linear models, but this time within all domesticated hosts, and within all wild hosts. We also calculated 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. Many SNPs exceeded the 99.9% threshold for domestication phenotypes as well as individual plant phenotypes (Figure R7). Domestication sensitivity often identified unique SNPs from domesticated or wild alone (Figure R8; Figure R9).

We annotated genes [from Bc genome annotation? Neurospora?] within 2kb of significant SNPs. [Something interesting about SNPs from overlaps in hosts… add later*]. [Need to correct the following. this includes repeat mentions of the same genes if >1 SNP per gene above 99.9% threshold: At the gene level, 30 genes were associated with domesticated, wild, and domestication sensitivity phenotypes, but ~200 genes were uniquely identified by a single phenotype (Figure R10)].* A total of 189 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 indicates 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.

DISCUSSION

Summary paragraph

**Domestication and altered pathogen virulence genetics**

These results provide evidence of a mild 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 pathogen interactions, it is not the primary evolutionary force in defining these interactions. In the generalist B. cinerea, however, domestication effects are small. We measured an 18% increase in susceptibility across domesticated varieties, but this effect was not statistically significant. 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 is only true for a subset of *B. cinerea* genotypes. If the effect of host domestication varies by *B. cinerea* genotype, we must study many genotypes to truly understand the factors contributing to *B. cinerea* virulence. Smaller sample sizes could miss the host domestication effect entirely, or provide a false positive signature of consistently increased 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 within domesticated lines, as new combinations of alleles are mixed together.

**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 [rough number per phenotype] contribute to *B. cinerea* virulence. This is in contrast to the few genes involved in quantitative virulence of specialist pathogens. Further studies can explore the question of whether fewer genes, of larger effect, contribute to virulence of *B. cinerea* on other hosts.

* + Test model using ~10 big SNPs for fx size on trait?

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 mechanisms or pathways. 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 varies 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. We also did not 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**

**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). 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 plant 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-G 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 host-sensitive isolates, F is 5 isolates collected from tomato tissue, G is 3 domestication-sensitive isolates.

Figure R5. *B. cinerea* lesion size is a polygenic trait on tomato.

Figure R6. Overlap in lesion size SNPs > 99.9% threshold across multiple host plant phenotypes. Chromosomes are differentiated by shading. Frequency is number of phenotypes in which the SNP exceeds the threshold.

Figure R7. Top 50 SNPs for lesion size phenotype on each host plant. Points are color coded by plant host.

Figure R8. Overlap in lesion size SNPs > 99.9% across individual-host phenotypes and domestication phenotypes.

Figure R9. Top 50 SNPs for lesion size for each domestication phenotype. Domestication sensitivity is (domesticated – wild / domesticated).

Figure R10. Venn diagram of SNPs identified >99.9% for each domestication phenotype.

Figure R11. Venn diagram of genes with a significant SNP identified >99.9% for each domestication phenotype.

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