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

**Nicole E. Soltis1, Susanna Atwell1, Gongjun Shi1,2, Rachel Fordyce1,3, Raoni Gwinner1,4, Daniel J. Kliebenstein1,5**

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

2Gongjun Current address

3Rachel current address

4Raoni Current address

5DynaMo 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**

**Introduction**

Pathogen lifestyle and host resistance

Pathogen virulence and host-plant susceptibility are highly complex traits. Virulence and susceptibility can be viewed as the cumulative outcome of interactions between host pathways and pathogen pathways. Specialist pathogens are only pathogenic (and therefore exhibit virulence) on a narrow range of hosts. Suitable hosts may be limited to a single species or genus, leading to coevolution between host and pathogen which allows crosstalk between genes contributing to pathogen virulence and genes contributing to host susceptibility. Generalist pathogens, in contrast, can affect diverse hosts across taxa. They may be less sensitive to variation in host phenotypes, including some resistance strategies. Most known genes for plant resistance to pathogens confer qualitative resistance through plant innate immunity. A common genetic basis of plant resistance involves 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 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 R gene strategy often induces programmed cell death, a strategy which is effective against biotrophic pathogens feeding on living tissue {Glazebrook 2005}. This gene-for-gene resistance depends upon specific recognition, and requires close coevolution between host and pathogen if the host is not responding to highly conserved pathogen signals. The reciprocal selective pressures present in interactions between hosts and specialist pathogens make evolution of these specific genetic interactions relatively common. In contrast, generalists respond to evolutionary pressures from many host species, making the evolution of gene-for-gene resistance unlikely.

It is unclear what effect domestication of host plants would have on R-gene mediated resistance. Domestication may lead to loss of some R-genes, or reduce diversity at those loci. Domestication is also expected to affect the path of coevolution between host and pathogen. Domestication poses a strong genetic bottleneck for many species. In theory the population bottleneck of plant breeding has led to a reduction in genetic diversity genome-wide, including regions contributing to pathogen resistance. Genetic variation contributing to pathogen resistance, and variation in resistance phenotypes, in domesticated plants is particularly likely to be low if pathogen pressures are reduced in cultivation. In contrast, persistent pathogen pressures throughout cultivation could select for resistance loci. Host domestication may affect the path of pathogen coevolution as well, likely more strongly in host-restricted specialists than in generalists. Cultivation practices such as greenhouse growth can affect the evolution of necrotrophic pathogens (Decognet, Bardin et al. 2009).

In plants, most naturally variable genes for generalist pathogen resistance likely contribute to quantitative, rather than qualitative, resistance. There are no known naturally variable large-effect resistance loci for plant defense against generalist pathogens. Further, there are no known naturally variable large-effect virulence loci in generalist pathogens. A few genes are known to contribute to quantitative plant resistance to pathogens. Genes involved in secondary metabolite biosynthesis regulate quantitative resistance (Ferrari, Galletti et al. 2007). Additional transporters and kinases contribute to resistance. To identify the genomic basis of resistance to generalist and necrotrophic pathogens, we must work with genetic variation within pathogens and their plant hosts.

**Selected pathogen-host system**

To look at the interactions between genetic variation in plants and pathogens and the role of evolutionary processes including differentiation by lineage and domestication, we chose to focus on *Botrytis cinerea*. *B. cinerea* causes major pre- and post-harvest crop losses in many species, in the field and greenhouse (Nicot and Baille 1996, Elad, Williamson et al. 2007). *B. cinerea* is an extreme generalist with evidence for quantitative resistance. Single isolates of *B. cinerea* exhibit extreme host ranges in contrast to other pathogens. *Fusarium oxysporum* is a fungal species which is pathogenic on diverse plant hosts. Many of the individual strains, however, are highly host specific (Katan 1999). In contrast, *B. cinerea* isolate B05.10 pathogenesis has been studied on *A. thaliana*, *Phaseolus vulgaris*, *Capsicum annuum*, *Solanum lycopersicum*, multiple wild *Solanum* *spp*., among other host species (Deighton, Muckenschnabel et al. 2001, Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007, Corwin, Copeland et al. 2016).

Virulence phenotypes and the underlying genetics are highly variable between *B. cinerea* isolates. Quantitative resistance to *B. cinerea* in *A. thaliana* appears to be largely isolate-specific; GWAS identified mostly non-overlapping sets of candidate loci for resistance to each of four *B. cinerea* genotypes (Corwin, Copeland et al. 2016). The plant response likely varies depending on molecular patterns perceived from individual pathogen genotypes. In specific studies of virulence mechanisms, production of the toxin botrydial affects virulence in only some isolates (Siewers, Viaud et al. 2005). The effect of jasmonates in inhibiting *B. cinerea* virulence on *A. thaliana* also varies by isolate (Rowe, Walley et al. 2010). Genes contributing to *B. cinerea* – host interactions affect a diversity of processes, but most studies do not look at whether these genes function in diverse roles across the pathogen genotypes, or how gene function varies across hosts.

Studies of plant resistance have identified several mechanisms contributing to quantitative plant resistance and *B. cinerea* virulence. The *B. cinerea* toxins botrydial and botcinic acid increase virulence on several host plants including tomato (Siewers, Viaud et al. 2005, Dalmais, Schumacher et al. 2011). VELVET is necessary for oxalic acid production and *B. cinerea* mutants exhibit reduced virulence on multiple hosts (Schumacher, Pradier et al. 2012). *B. cinerea* also has virulence genes for cell wall degradation in the plant (ten Have, Mulder et al. 1998). There is no evidence for qualitative resistance to *B. cinerea* (Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016), and previous studies indicate quantitative resistance in the response of *Arabidopsis thaliana* to *B. cinerea*, due to an interaction between plant host genotype and isolate genotype {Corwin 2016}.

Tomato is one of the numerous hosts to *B. cinerea*, in which it causes major crop loss due to both pre- and post-harvest infection. Resistance to *B. cinerea* is a quantitative trait in tomato. QTL have been identified for *Solanum* susceptibility to *B. cinerea*, explaining up to 15% of phenotypic variation in a stem bioassay (Finkers, van Heusden et al. 2007). There is evidence for quantitative resistance to *B. cinerea* in the closest wild relative to tomato as well as other *Solanum* species, though this has not been directly tested in comparison to domesticated *S. lycopersicum* (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002). Further, tomato domestication has altered genetic variation for the circadian clock phase {Muller 2016}, which likely contributes to modulation of pathogen resistance. In *A. thaliana*, multiple genes incorporate signaling from the circadian clock and pathogen attack {Sauerbrunn 2003; Bhardwaj 2011; Weyman 2006}, suggesting connections between these response pathways.

The effect of domestication on plant-pathogen interactions is largely untested. Domestication poses a strong genetic bottleneck, reducing diversity genome-wide. We assume that this extends to pathogen resistance loci; resistance alleles are likely lost during the domestication bottleneck. This loss of resistance is assumed to extend to all domesticated varieties. Further, selective pressures from pathogens may be reduced under cultivation. In contrast, domesticated plants may experience increased selective pressures from some pathogens. *B. cinerea* causes extensive preharvest damage in *S. lycopersicum* cultivation, so it is unclear what the effect of domestication will be on plant susceptibility and pathogen virulence.

In this study, we are conducting GWA in the pathogen to see how it broadly handles host phenotypic variation. 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). We inoculated individual tomato leaflets from 6 domesticated varieties of *S. lycopersicum*, and 6 wild accessions of *S. pimpinellifolium* with spore suspensions of 91 *B. cinerea* isolates. We asked whether susceptibility to *B. cinerea* depends on pathogen genotype or tomato host genotype, and whether the same loci confer *B. cinerea* virulence across host genotypes. In our analysis of lesion images at 72 hours post inoculation, both host and pathogen genotype contribute to virulence. However, we found no significant interaction between host and *B. cinerea* genotype species-wide. We also find no species-wide evidence of a significant domestication effect upon *B. cinerea* virulence, though domesticated varieties are slightly more susceptible on average. This suggests that individual isolates are generalists across tomato genotypes and across domestication in *Solanum.* A subset of single isolates, however, are sensitive to tomato domestication. All three of these show increased virulence on domesticated tomato varieties. No isolates are significantly affected by individual tomato genotypes. Further, lesion size is more variable on domesticated than wild genotypes, in contrast to the expected reduction in variation at resistance loci following the domestication bottleneck. 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: 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]

**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.

We took digital photos of all leaflets at 24, 48, and 72 hours post inoculation for downstream 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 generalized linear model for the full experiment, including the fixed effects of isolate, plant domestication, plant genotype (nested within domestication), the random effects of experiment, and the interaction effects of experiment with isolate and experiment with plant.

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. We used SNPs from (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.

**Results**

**Experimental Design**

We wanted to directly measure the impact of tomato domestication and genetic variation on quantitative resistance. To measure quantitative resistance, we infected tomato leaflets with a collection of 91 diverse *B. cinerea* isolates. *B. cinerea* is an endemic necrotroph, and host resistance to this generalist pathogen is quantitative, with no evidence of qualitative defense loci (Rowe and Kliebenstein 2008, Corwin, Copeland et al. 2016). Previous studies have examined the contrast in *B. cinerea* resistance between wild and domesticated tomato using distantly related species such as *S. chilense* (Nicot, Moretti et al. 2002, Ten Have, van Berloo et al. 2007), *S. chmielewskii* (Nicot, Moretti et al. 2002), *S. habrochaites* (Finkers, van Heusden et al. 2007, Ten Have, van Berloo et al. 2007), *S. hirsutum* (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002), *S. lycopersicoides* (Guimaraes, Chetelat et al. 2004), *S. neorickii* (Ten Have, van Berloo et al. 2007, Finkers, Bai et al. 2008), *S. peruvianum* (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002), *S. pennellii* (Nicot, Moretti et al. 2002) and *S. pimpinellifolium* (Egashira, Kuwashima et al. 2000, Nicot, Moretti et al. 2002). 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. We selected tomato genotypes including 6 domesticated *Solanum lycopersicum* cultivars and 6 wild *S. pimpinellifolium* genotypes. 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 grown to completely consume infected leaflets. Lesion area is a composite phenotype from the interaction of host and pathogen genetics that has been utilized in a number of studies on the molecular and quantitative genetic basis of plant-*Botrytis* interactions (Rowe and Kliebenstein 2008).

We performed statistical analysis of lesion size with a generalized linear model (GLM). Within the model, we tested the fixed effects of isolate genotype, plant species (domesticated or wild), plant genotype (which is nested within species), and position of sampled leaflet (apical or basal). We also considered the random effects of experiment, block (nested within experiment), individual plant, and individual leaf (nested within sample plant). The terms for individual plant, leaf, and leaflet position did not significantly improve the model, so we omitted them from further analysis. Our final model also included the interaction terms of isolate by plant species, and isolate by plant genotype (nested within species). The final model shows that genetic variation within both the host plant and the pathogen affect lesion growth (Table R1). Domestication also impacted lesion formation, as shown by the significant effects of tomato genetic variation between domesticated and wild species. We did not find evidence for significant interaction effects between isolate and plant genotypes. This may have been due to the vast number of degrees of freedom, as the isolate-plant interactions contribute a large proportion of the variance in lesion size (Table R1).

**Domestication and lesion area**

Existing literature, largely studying qualitative resistance to biotrophic pathogens, has proposed that domestication increases susceptibility to pathogens and decreases plant genetic variation for disease resistance due to selection bottlenecks during domestication.

In agreement with domestication theory, lesion size is slightly greater on average (18% increase) on domesticated tomato compared to wild tomato (p <2e-16, Table R1) (Figure R2).

In contrast to theory, the domesticated tomato genotypes had a wider range of average lesion formation than wild genotypes (5% to 95% interval: 2.03 cm2 range on domesticated, 1.76 cm2 range on wild). 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. 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). Overall, we see evidence for a slight domestication impact on *Botrytis cinerea* defense, but this depends on host genotype.

**Host variation and lesion area**

Domestication does impact lesion size, but most variance is due to genetic variation in the isolate and in host plant genetics (Table R1).

**Pathogen**

Our 91 B. cinerea genotypes were isolated from various eudicot plant hosts, including tomato stem tissue (2 isolates; T3, KT) and tomato fruit (3 isolates; KGB1, KGB2, Supersteak)

Isolates collected from tomato stem or fruit tissue are not among the most virulent group (Figure R4F). For *B. cinerea* genotypes isolated from tomato tissue vs. other hosts, there is no significant difference in lesion size across all hosts on either domesticated (t-test; t=-1.10, 4.3 df, p=0.330) or wild (t-test; t=-1.09, 4.2 df, p=0.332) tomato. In fact, one isolate collected from tomato tissue (KGB1) is within the 10 least-virulent isolates (Figure R4F). This may suggest a generalist strategy for individual isolates, due to this apparent lack of host-specificity.

**Host-pathogen interactions**

**Domestication**

We also tested whether genetic differences between wild and domesticated hosts interact with pathogen genetics to determine lesion size. Lesion size varies across host for many of the isolates, suggesting an interaction between the genomes of *B. cinerea* and tomato (Figure R4). However, domestication did not have a significant interaction effect with isolate genotype (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).

**Plant genotype**

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 and plant genotype nested within domestication, and the random effect of experiment. Through this single-isolate GLM analysis, a subset of isolates show a significant (p < 0.05) interaction with host genotype (Figure R4E).

**BigRR/GWAS**

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 [BigRR citation] to calculate GWA between B. cinerea SNP variation for the 92 isolates and lesion size. To determine significance of SNP effects, we permuted phenotypes 1000x to calculate 95, 99, and 99.9% thresholds within each plant host.

On 3 of the domesticated and 5 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 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 very large effect sizes on multiple hosts.

We also 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 ran bigRR for each of these 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

Ideas:

Multiple lines of evidence support a contrast between the genetic basis of pathogen virulence in specialists and the generalist, Botrytis cinerea.

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]. 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 specialists, 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.

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.

Further, the genetics of B. cinerea virulence do not conform to our expectations based on the genetics of virulence in specialist pathogen studies. We did not identify any MAMPs or PAMPs as major loci contributing to virulence across tomato varieties.

* No MAMPs/PAMPs identified
  + No chitin, glycans, glycolipids…
  + No mannans (JAC + Klieb)
* Not caused by a few genes of large effect
  + Effect size of SNPs small (~1 mm?)
  + MANY SNPs
  + Sum up SNP fx of top hits?
  + Test model using ~10 big SNPs for fx size on trait?

**FIGURES**

Figure R1. Will be an image of the detached leaf assay and leaf/ lesion calls.

Figure R2. Violin plots of lesion size due to Botrytis cinerea growth on tomato host genotypes. Plots include all replicates of lesion size measurements across isolates.

Figure R3. Interaction plot for domestication. Each line traces the average lesion size for a single Botrytis isolate.

Figure R4. Interaction plot of lesion size due to individual Botrytis cinerea isolates on tomato host genotypes. Each line traces the average lesion size across plant genotypes for a single Botrytis isolate. A is all isolates, B is B05.10, C is the top 10 highly-virulent isolates, D is the bottom 10 saprophytic isolates, E is host-sensitive isolates, F is 5 isolates collected from tomato tissue, G is 3 domestication-sensitive isolates.

Figure R5. 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 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 > 99.9% across individual-host phenotypes and domestication phenotypes.

Figure R8. Top 50 SNPs for lesion size for each domestication phenotype. Domestication sensitivity is (domesticated – wild / domesticated).

Figure R9. Venn diagram of SNPs identified >99.9% for each domestication phenotype.

Figure R10. 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.

Decognet, V., M. Bardin, Y. Trottin-Caudal and P. Nicot (2009). "Rapid change in the genetic diversity of Botrytis cinerea populations after the introduction of strains in a tomato glasshouse." Phytopathology **99**(2): 185-193.

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.

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.

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.

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.

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.

Rowe, H. C., J. W. Walley, J. Corwin, E. K.-F. Chan, K. Dehesh and D. J. Kliebenstein (2010). "Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in Botrytis cinerea pathogenesis." PLoS Pathog **6**(4): e1000861.

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.

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.

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.