A generalist pathogen view of plant evolution

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Abstract

Plant-pathogen interactions are largely modeled as co-evolutionary arms races based on specialist pathogens. Less is known about how generalist pathogens interact with diverse hosts. Here, we use a collection of 98 isolates of *Botrytis cinerea* to address how this generalist necrotroph perceive plant evolution across 90 plant genotypes from eight Eudicot species. We show that interactions in this pathosystem are largely defined by the plant species with small and inconsistent effect of plant domestication. More surprisingly, plant susceptibility to *Botrytis* shows little association to evolutionary distances between the plant species. We also show that *Botrytis* virulence and host specificity is polygenic with GWA associated genes covering 12% of Botrytis gene transcript.

Plant-pathogen interactions influence ecosystems by altering the diversity and structure of natural plant communities. They also impose significant yield losses on agriculture. All these interactions are influenced by the interaction of allelic diversity between genes in plant resistance and pathogen virulence. Plant resistance is composed of innate and inducible immune systems that recognize danger and mount physiological, physical and chemical responses that defend against single strains to entire families of pathogens (Jones & Dangl 2006). To counter these plant defenses, pathogens have evolved diverse mechanisms to attack and/or interfere with the plant including virulence factors and toxins (Rodriguez-Moreno *et al.* 2018). The genetic diversity of the plant and pathogen mechanisms are dynamically shaped by the evolutionary histories of how the plant and pathogen species interact with each other and their changing environment (Gilbert & Parker 2016; Velásquez *et al.* 2018).

As a complication to a universal model of plant/pathogen co-evolution, plant pathogens have a wide range of host specificity and life styles, ranging from obligate biotrophs, parasites that survive within living host cells and develop precise, intricate interactions with their hosts, to generalist necrotrophs that can kill a wide range of host plants (Möller & Stukenbrock 2017). Plant-specialist biotrophs are considered to follow a co-evolutionary arms-race evolutionary model where qualitative virulence is linked to gene-for-gene specialization. Similar host-pathogen specificity model is in accord with *Fusarium oxysporum* and *Alternaria alternata,* generalist pathogens where individual strains show host-specificity that is contained on disposable chromosomes (Bertazzoni *et al.* 2018; Meena *et al.* 2017; van der Does & Rep 2007). How applicable the co-evolutionary arms race model is to generalist pathogens such as *Botrytis cinerea* (a.k.a grey mold; *Botrytis* hereafter) remains to be determined. Botrytis is a pan-global necrotrophic fungus in which individual isolates can infect hundreds of plant species, from mosses to gymnosperms (Fillinger & Elad 2015). It causes billions/annum of crop damage both pre- and post-harvest to various crops from ornamentals to vegetables and fruits (Veloso & van Kan 2018). The ability of Botrytis isolates to infect diverse plant orders has been linked to its high standing genetic variation (Atwell *et al.* 2018; Atwell *et al.* 2015), the production of diverse small RNAs released to silence host defenses (Weiberg *et al.* 2013) and an arsenal of enzymes used for host tissue penetration and toxic proteins/metabolites (Nakajima & Akutsu 2013). In this system, low host specificity means that individual isolates are evolving against a wide range of host plants and suggests the need to develop a different paradigm for how these systems co-evolve or shape either the hosts resistance or the pathogens virulence (Corwin & Kliebenstein 2017).

Current plant pathology models based on specialist pathogens predict a nested pattern of virulence and specialization that follows plant evolutionary distances (Gilbert & Parker 2016)(Schulze-Lefert & Panstruga 2011). In this model, resistance against a common pathogen would be highly similar between closely related species and slowly decay with the evolutionary distance between species. The evolution of defense mechanisms yields a more complicated picture. Some host defense components such as resistance genes (Jacob *et al.* 2013) or specialized defense compounds (Chae *et al.* 2014) are specific to limited lineages or even singular plant species. In contrast other components such as cell walls (Sørensen *et al.* 2010), defense hormone signaling (Berens *et al.* 2017) or reactive oxygen species (Inupakutika *et al.* 2016) are widely shared across plant lineages. Further complicating this model is that, crop domestication affects co-evolutionary dynamics associated to a reduction of plant defense compounds and through a loss of genetic diversity in large-effect resistance genes (Chen *et al.* 2015)(Karasov *et al.* 2014). To date, there has not been a coordinate assessment of how host/pathogen interactions are affected across host evolution. *Botrytis* isolates ability to infect most plants yields the opportunity to empirically measure how host defenses have evolved across plant lineages and domestication by measuring their effect on a single collection of a generalist pathogen. This study addresses how a generalist pathogen interplays with plant evolutionary histories to test several assumptions formulated using co-evolutionary theory developed using host/specialist pathogen interactions.

Using a randomized replicated design we infected 98 isolates (Table S1) onto the leaves of 90 genotypes (Table S2) representing 6-12 genotypes from each of eight different eudicot plants for a total of 51,920 independent lesion measurements. As a common measure of the host-pathogen interaction, we utilized lesion area on detached leaves. Lesion area is a heritable estimate of the interaction of plant resistance with fungal virulence (Corwin *et al.* 2016; Soltis *et al.* 2018; Zhang *et al.* 2017). The *Botrytis* isolate collection contains extensive genetic diversity that has been highly shuffled by recombination creating an admixed pool of virulence mechanisms (Atwell *et al.* 2018)(Table S1). To test the relative influence of lineage evolution and domestication, we used seven distinct crop eudicot species that sample both natural eudicot evolution and also the influence of human domestication within each species (see Material and Method). Within each plant species, six genotypes represented the high improvement germplasm (cultivar, inbred lines) and six represented the low improvement germplasm (wild, landraces), allowing us to compare the evolution between eudicot lineages to the effect of short-term evolutionary modifications

under artificial selection. As a reference comparison, we also measured the virulence of the isolate collection on five single genes mutant of *Arabidopsis* with compromised defense mechanisms (Rowe *et al.* 2010; Zhang *et al.* 2017).

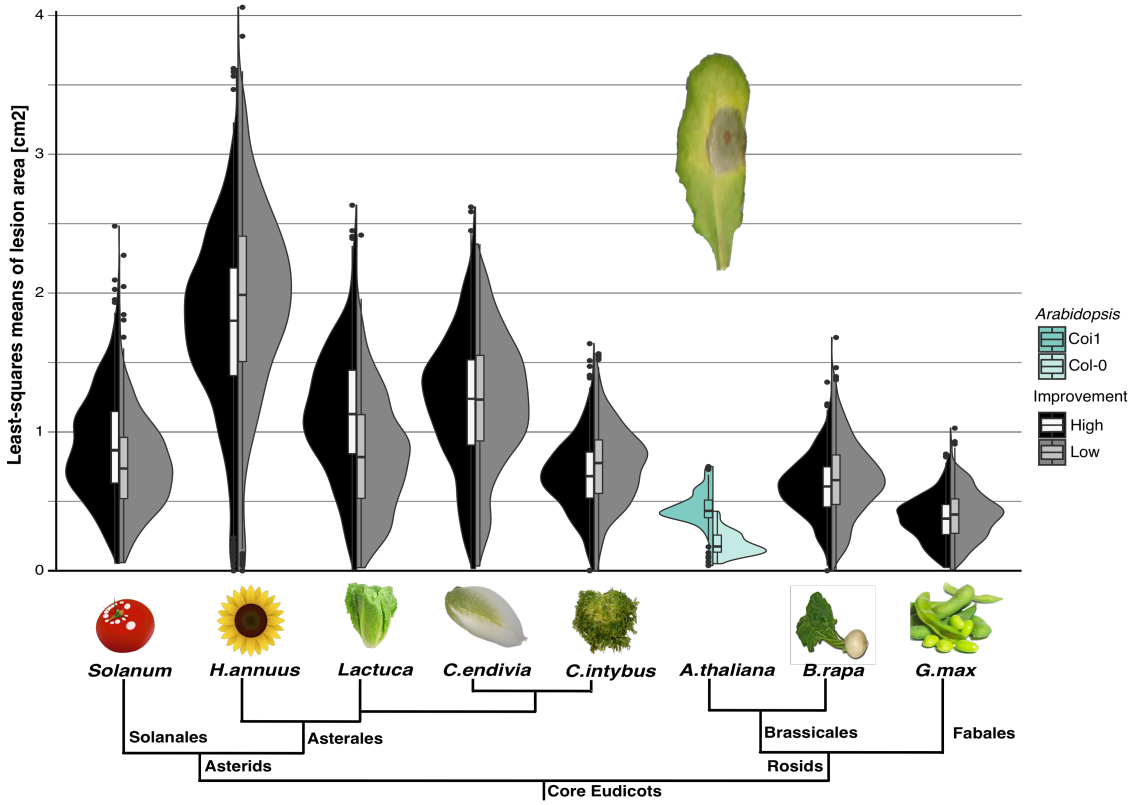

Figure 1: Least-squares means of leaf lesion area at 72 hours post infection for eight eudicot species. Half-violin and boxplot (median and interquartile range) represent the lesion area distribution for genotypes with high (black) and low (grey) level of improvement. For *A. thaliana*, wild-type (Col-0) and jasmonic acid signaling mutant (*coi1*) are represented. The non-scaled tree represents the phylogenetic relationship between species. An example lesion on an *A. thaliana* leaf is also provided.

The eight eudicot species present a wide distribution of susceptibility with mean lesion area ranging from no visible damages to over 4cm^2 of necrotic leaf tissue after 72 hours of infection (Figure 1). *A. thaliana* is the eudicot species with the lowest susceptibility while *H. annuus* is the most susceptible species (Fig. 1). Within each species, the effect of variation between the *Botrytis* isolates (40-71% of variance) and their interaction with the host genotype (15-35% of variance) controls the vast majority of the variance (Fig. 2B, Fig S2B). A meta-analysis across all the seven crop species showed that differences in susceptibility to *Botrytis* between species is the

main determinant of lesion area accounting for 52% of the total variance (Fig. 2A). Further, the interaction between the host species and isolates (16% of total variance) matters more than the isolates alone (12% of total variance). As such, the host-Botrytis interaction is controlled by genetic variation between and within plant species and the interaction with pathogen genotype.

For each crop species, high and low improvement genotypes were included to test how domestication may have influenced susceptibility to *Botrytis*. Domestication is generally modeled to decrease gene-for-gene mediated resistance to specialist pathogens (Karasov *et al.* 2014). In contrast to specialist pathogen, high improvement genotypes are more resistant to *Botrytis* than wild genotypes for five species (sunflower, endive, chicory, *Brassica* and soybean) (Fig. 1). Only two species, tomato and lettuce, showed decreased resistance in high improvement genotypes (Fig. 1). While the effect of crop improvement is statistically significant, the overall effect on susceptibility to *Botrytis* is exceedingly small, 0.6% of the total variance across all plant species and 2.1-4.3% of variance within specific species (Fig 2). This shows that domestication does not reduce variation in lesion area or have a universal directional effect on resistance to the generalist Botrytis. This is regardless of whether the domestication event targeted seed, fruit or leaf (Fig 2, Material & Method).

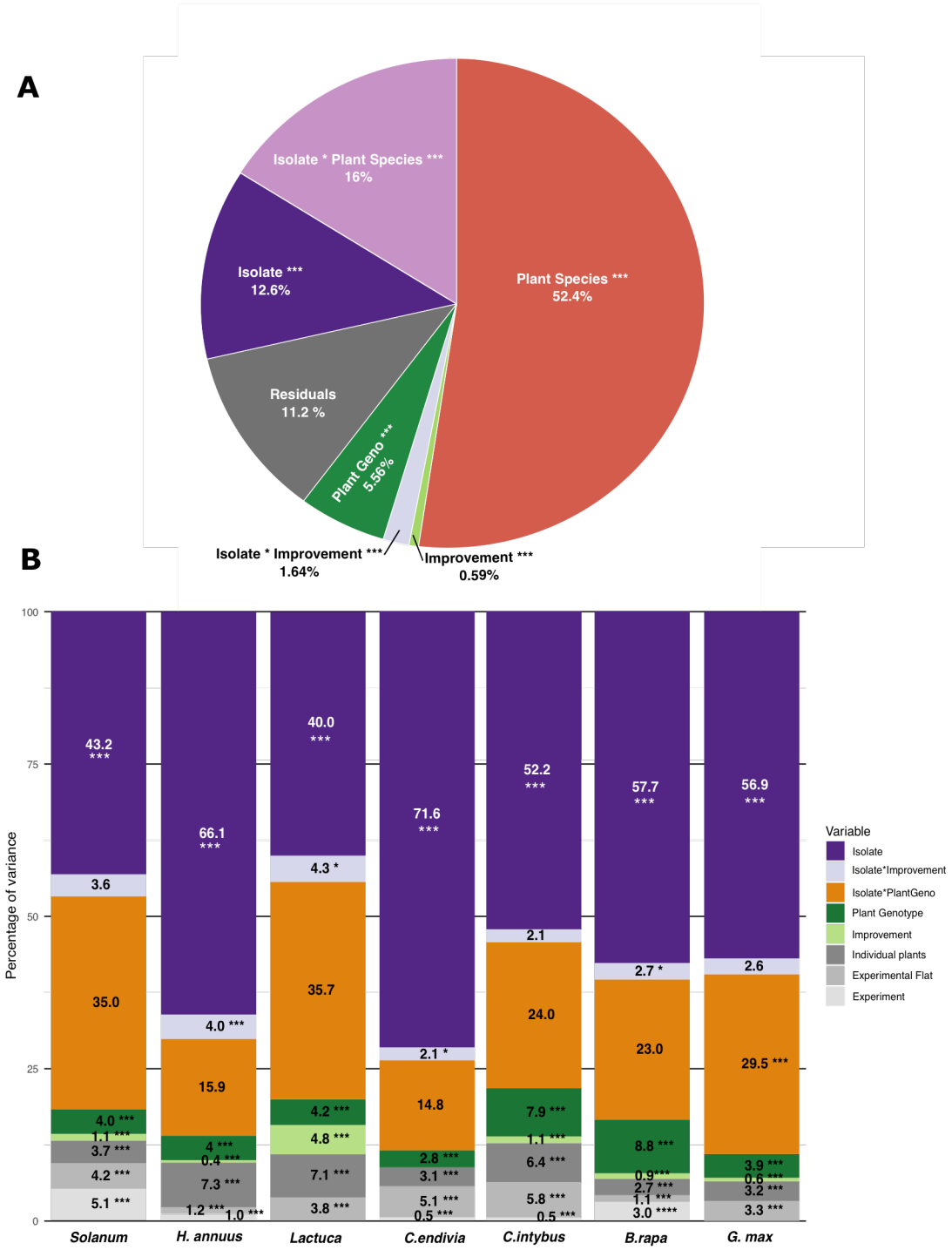

Figure 2: A) Meta-analysis linear model estimating the contribution of plant species, plant genotypes, level of improvement, Botrytis isolates and their interaction on the percentage of variance in lesion area. B) Species-specific anova of linear mixed models that estimate the percentage of variance in lesion

area. In grey are the experimental parameters used as random factor. Significance: *p<0.05, **p<0.01, ***p<0.005

Current plant pathology models assume that relatedness between host species correlates to relatedness in the patterns of pathogen susceptibility (Gilbert & Parker 2016; Schulze-Lefert & Panstruga 2011). To test this assumption, we utilized the standardized mean lesion area of every *Botrytis* isolate on every plant genotype to test the relatedness between host species for the interaction. This showed that the host-pathogen variation properly identifies species groupings for all species. Beyond the individual species level, the relatedness between hosts did not correlate with the relatedness between the measured host-pathogen interactions (Fig.3). For example, evolutionarily distant species such *B. rapa* and lettuce have a similar susceptibility pattern using the 98 *Botrytis* isolates. In contrast, the two sister species, *C. endivia* and *C. intybus*, have highly divergent susceptibility patterns. Thus, the interaction of *Botrytis* with host plants is predominantly defined by variation at the species-by-species level with minimal extension to the level of host plant family or genus.

To provide a molecular benchmark for how the host-pathogen relationships measured across the eudicots compare to single large effect alterations in plant defense, we included data for *Arabidopsis thaliana* Col-0 and five single gene knockout mutants (coi1, anac055, npr1, tga3, pad3) (Rowe *et al.* 2010; Zhang *et al.* 2017). These mutants have a large effect on plant susceptibility (39% of the variance in lesion area, Fig. S2) in comparison to variation in crop genotypes (Fig. 2). While these mutants are considered to abolish major sectors of the immune response, the *Botrytis*-host interactions still identifies all the mutations in salicylic acid, jasmonic acid signaling and phytoalexin production as representatives of Arabidopsis and not as random data points in the tree. Thus, *Botrytis* is interacting on a species level with the eudicots and this is not inherently constrained by individual defense signaling pathways or phytoalexins.

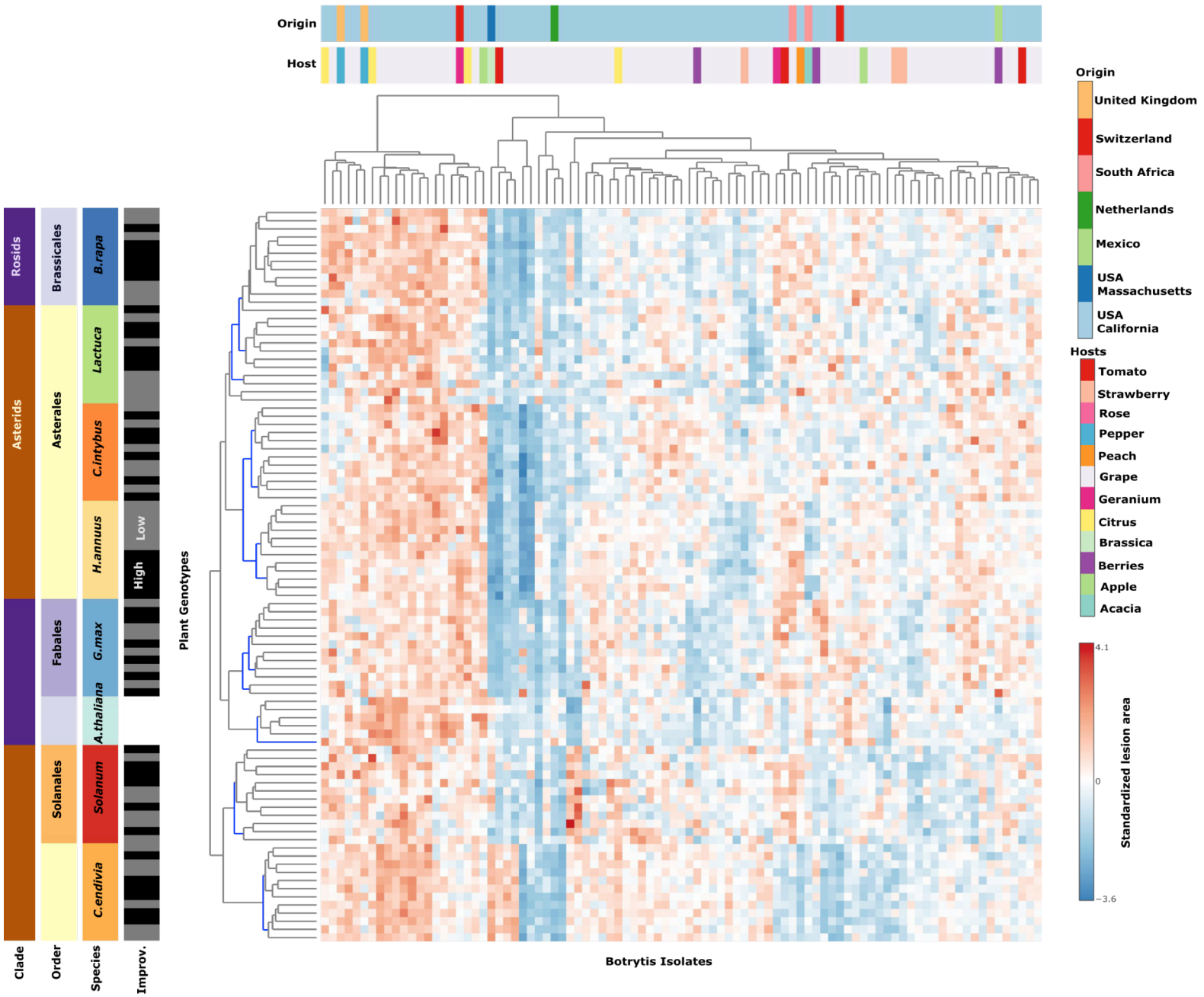

Figure3: Heatmap of standardized (z-scored) least-squares means of lesion area for *Botrytis* isolates (x-axis) interacting with 90 plant genotypes (y-axis). The isolates were isolated largely in California (light blue color in the origin bar) and on grape (light purple in the host bar). For *A. thaliana,* five single gene knockout mutants and the corresponding wild-type Col-0 are presented. For the seven crop species, six genotypes with low (grey) and six with high (black) level of improvement are presented. The seven crop species were chosen to represent a wide spectrum of phylogenetic distances across rosids (Brassicales and Fabales) and asterids (Asterales and Solanales). Branches in the dendrogram that are supported with 95% certainty after bootstrapping are indicated in blue.

How pathogen's virulence behaviors evolve along the generalism-specialism continuum remains largely unknown (Barrett et al 2009). Specialism is often described as an endgame, with some empirical evidences linking high virulence with adaptation to host and therefore high host-specificity (Leggett *et al.* 2013). The diversity of virulence patterns across eight eudicot species in the *Botrytis* isolates (Figure 3) constitutes a unique dataset to estimate host specificity and overall virulence within a generalist pathogen. While the isolates cover a range of host specificity and virulence (Fig.4), the host it was collected from and the geographical origin do not provide structure to the patterns. B05.10, the reference strain for the *Botrytis cinerea* genome and the isolate used in >90% of all papers to assess susceptibility to *Botrytis*, is the isolate with the lowest specificity/highest generalist behavior (Fig. 4). In our data, isolates with increased host specificity had on average lower virulence both across all eudicots and on their preferred hosts (Fig. 4). Further, isolates with increased host-specificity were relatively rare in the population. In combination, this suggests that *Botrytis* is under pressure to maintain broad host ranges and moderate virulence within individual isolates.

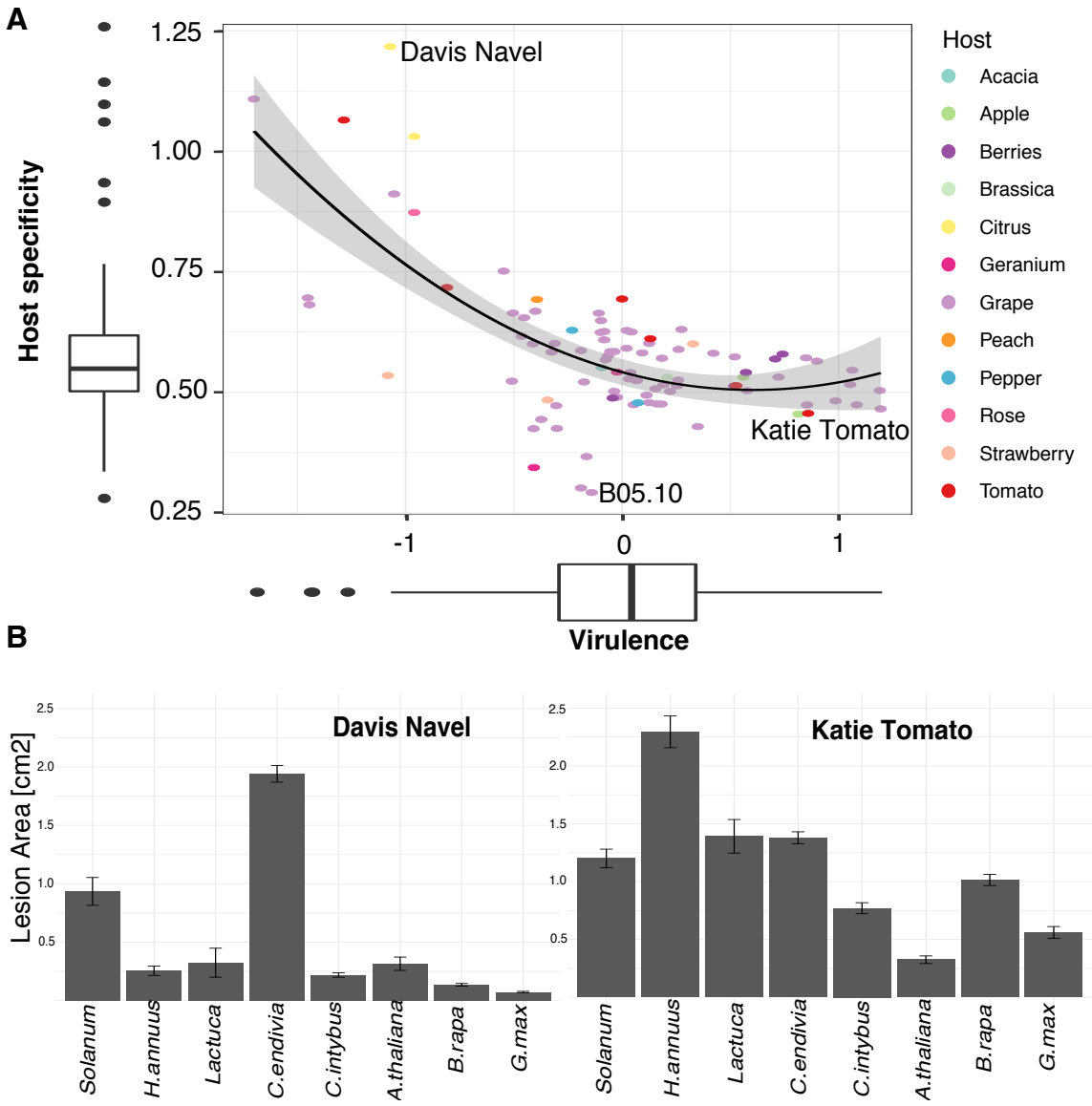

Figure 4: A) Estimates of overall virulence across eight eudicot and host specificity for 98 isolates of *Botrytis cinerea* colored according to the plant host they were collected from. Quadratic curve (R^2=0.44, p-val=6.36E-13) and its confidence interval (in grey) are plotted. B) The averaged lesion area and standard error across the eight species is provided for two isolates at the extremes of the host specificity/virulence distribution.

While the genomic architecture of virulence and host specificity in specialist pathogens is defined by few large effect loci, it remains largely uncovered in *Botrytis*. Genome-wide Association Study (GWAS) reveal that both overall virulence across eight eudicots and host specificity are highly polygenic traits with respectively 4351 and 5705 significant SNPs at a conservative 99.9%

threshold (Figure 5). All of these significant SNPs are spread across 16 of the 18 chromosomes and are of small effects. The chromosome 17 and 18, hypothesized to be potential disposable chromosomes (Bertazzoni *et al.* 2018) are not associated with virulence or host specificity (Figure 5). This suggests that at contrary to generalist pathogens with high host specificity such as *Alternaria* or *Fusarium*, *Botrytis* doesn't have specific genomic structures for host specificity. The significant SNPs are located in 1479 genes associated with virulence and 1094 genes associated with host specificity (Table S3), which considered together represent 12 % of the gene transcript of *Botrytis*. The genetic architecture of virulence and host specificity is largely different with 9.34% of SNPs and 17.5% of genes that overlap between the two traits. Botrytis genome annotation is not complete enough to provide significance on gene ontology. However, the gene list includes 442 proteins (Table S3) identified in the *Botrytis* secretome (49.3% of the secreted proteins successfully identified in the B05.10 genome), suggesting the importance of secreted proteins in the successful virulence across plant species (Gonzalez-Fernandez et al. 2015).

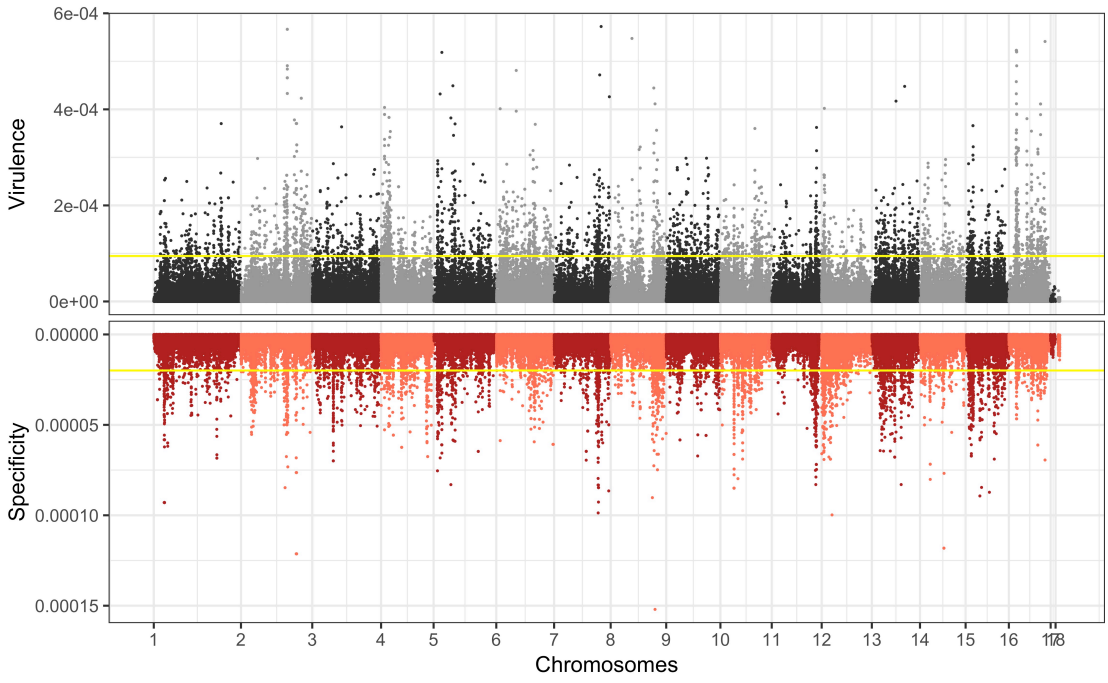

Figure 5: Effect size of 271,749 SNPs with reference to B05.10 genome estimated through ridge regression GWAS. In grey is plotted the effect on general virulence and in red is the effect on host specificity. The yellow lines represent the conservative significance threshold at 99.9% for each trait as determined by permutation.

The co-evolutionary arms-race model of specialist plant-pathogen interaction create a coherent explanation for how large-effect virulence and resistance genes evolve within most pathosystems. Further, in such systems, interaction specialization is the most successful strategy, with generalism being selected against in natural environments due to the exponential cost of adaptation to evade innumerable host receptors (Barrett *et al.* 2009). The data obtained by using Botrytis across a collection of dicots clearly shows that this evolutionary model is not a good fit for how host-generalist pathogens such as Plant-*Botrytis* may evolve. Empirically measuring how a population of *B. cinerea* interacted with multiple genotypes from eight plant species across four plant orders generated several observations. First, specificity in the host-*Botrytis* interaction is largely defined by the plant species with little relationship to evolutionary distances between the plant species. Second, the vast majority of *Botrytis* isolates are generalists with only a few individuals showing any propensity to prefer an individual host species. This shows that host-generalist interactions are highly responsive to variation within a host species and that the pathogen appears to be under pressure to maintain a generalist life style. One potential model to explain these observations is that a generalist pathogen uses extensive standing variation with recombination to provide the potential to rapidly adapt to any new defense mechanisms that may arise within a host species to defeat a specialist pathogen. This suggests a potential interplay between the evolution of specialist-host and generalist-host interactions. Combining this with previous work showing these interactions are highly polygenic in the host and the pathogen and involve complex transcriptome interactions shows that any model of how host-generalists evolve will require a genome wide understanding of resistance and that single gene models will not suffice (Corwin et al. 2016)(Zhang et al. 2017, 2018). As this work focused on the leaf, it raises the open question of how this image may change if alternative organs (stem, leaf, fruit) were used in each of the species. As defense mechanisms are developmentally programmed, it is possible that developmental variation could be as critical as variation between plant species. This illustrates the need to understand how rapidly generalist pathogens can adapt to new defense mechanisms that they may encounter in the wild, and the need to develop new genome level models of co-evolution in a complex environment.

Materials and Methods

Plant material and growth condition

Seven species were chosen to represent a wide diversity of phylogenetic distance, geographical origin and histories across the core eudicot (Fig. 1, Fig. S1, Table S1). For simplicity in the language, we refer to the seven different crops as 'species' although referring to taxa would be more appropriate for tomato and lettuce for which we selected sister species for wild and domesticated genotypes. This assay considers four Asteraceae species (*Helianthus annuus*, *Cichorium intybus*, *Cichorium endivia*, *Lactuca*), one Solanaceae (*Solanum*), one Fabales (*Glycine max*) and two Brassicales (*Brassica rapa, Arabidopsis thaliana*) (Table S2). *Arabidopsis thaliana* data was used as a reference for genotypes with altered plant susceptibility (Fig S2). This reference dataset is composed of Col-0 and five knockout mutants altering plant immunity (Zhang et al. 2017, Atwell et al. 2018), through the jasmonic pathway (anac055, coi1), salicylic pathway (npr1, tga3) and camalexin pathway (pad3), an anti-fungal defense compound in *Arabidopsis*.

While the chosen species partially represent the eudicot phylogeny (Fig1), they were also chosen to represent the diversity of plant domestication syndromes and geographical origins (Meyer *et al.* 2012) (Figure S2). *H. annuus* and *G. max* were domesticated for seeds, *Solanum* (tomato) for fruit (Lin *et al.* 2014) while *Lactuca*, *C. intybus* and *C. endivia* were domesticated for leaf and root (Dempewolf *et al.* 2008). All of these species are single domestication events while *B. rapa* domestication is more complex (Bird *et al.* 2017). *B. rapa* was domesticated and re-selected multiple times for multiple traits including seed, leaf and root morphology. For each species, twelve genotypes were selected, including six genotypes with low (wild or landrace) and six with high (cultivar, inbred lines) level of improvement. The genotypes were chosen based on description of domestication status and phylogeny for each species (Blackman *et al.* 2011) (Bird *et al.* 2017; Dempewolf *et al.* 2008; Liang *et al.* 2014; Lin *et al.* 2014; Valliyodan *et al.* 2016; Walley *et al.* 2017). For Lettuce, *Lactuca sativa* was sampled for high improvement and *Lactuca serriola* for low improvement accessions (Walley *et al.* 2017). For tomato, *Solanum lycopersicum* was sampled for high and *Solanum pimpenellifolium* for low improvement accessions (Lin *et al.* 2014; Soltis *et al.* 2018). For soybean the comparison was within *G. max* as the growth behavior, vining, and growth conditions, tropical, for wild soybean, *G. soja*, was sufficiently different as to unnecessarily confound the comparison.

C. endivia, *C. intybus*, *B. rapa*, *G. max and A. thaliana* seeds were directly sowed in standard potting soil. *Solanum* and *Lactuca* seeds were bleach-sterilized and germinated on wet paper in the growth chamber using flats covered with humidity domes. After 7 days, the seedlings were transferred to soil. Seed surface sterilization and scarification was used for *H. annuus* to increase seed germination. Seeds were surface sterilized in 30% bleach for 12 minutes, followed by rinsing with sterilized distilled water, and then soaked in sterilized water for 3 hrs. ¼ of the seeds were cut off from the cotyledon end, then placed in 100 mg/L Gibberellic acid for 1 hour, followed by rinsing several times with sterilized distilled water. After that, seeds were put in covered sterilized Petri dishes with wet sterilized germination disks at 4°C for 2 weeks, then sowed.

All plants were grown in growth chambers in pots containing Sunshine Mix#1 (Sun gro Horticulture, Agawam, MA) horticulture soil at 20°C with 16h hours photoperiod at 100-120 mE light intensity. All plants were bottom-watered every two days with deionized water for the first two weeks and then with nutrient solution (0.5% N-P-K fertilizer in a 2-1-2 ratio; Grow More 4-18-38). Experiments were conducted on mature fully developed leaves collected on adult plants that grew in these conditions for four to eight weeks (Table S4) to account for the different developmental rates. As it is challenging to fully compare developmental stages across species (soybean stages are defined by nodes, sunflower by leaf size and so on), all leaves for the assays were collected on plants in the vegetative phase at least a week before bolting initiation to minimize ontogenetics effects.

Botrytis collection and growth condition

This study is based on a collection of 98 isolates of *Botrytis cinerea.* The collection samples the *B. cinerea* isolate diversity across fourteen plant hosts and in smaller degree across geographical origins. Ninety percent of the isolates were isolated in California, largely in vineyards (70% of isolates were collected on grape), while the remaining 10% of the collection are worldwide isolates (Table S2). Although a large proportion of the isolates were collected in California, the isolate collection enfolds a large genetic diversity (Atwell *et al.* 2018). The spore collection is maintained for long-term preservation as conidial suspension in 30% glycerol at -80°C. The isolates were grown for each experiment from spores on peach at 21°C for two weeks.

Detached leaf assay

To maximize the comparability across such a diverse collection of wild relatives and crop species domesticated for different trait, leaves were chosen as a

common plant organ. Detached leaf assays were conducted following (Corwin *et al.* 2016). The detached leaf assay methodology has been used for testing plant susceptibility to plant pathogens in more than 500 scientific publications and is considered a robust method when coupled with image analysis. In brief, leaves were cut and added to trays in which 1cm of 1% phyto-agar was poured. The phyto-agar provided water and nutrients to the leaf that maintained physiological functions during the course of the experiment. *Botrytis* spores were extracted in sterile water, counted with a hemacytometer and sequentially diluted with 50% grape juice to 10spores/ul. Drops of 4ul (40 spores of *Botrytis*) were used to inoculate the leaves. From spore collection to inoculation, *Botrytis* spores were maintained on ice to stop spore germination. Spores were maintained under agitation while inoculating leaves to keep the spore density homogeneous and decrease technical error. The inoculated leaves were maintained under humidity domes under constant light. Pictures of the trays were taken every twenty-four hours to follow the progression of lesions. After 24h most of spores germinated, at 48h most isolates are well developed within the leaf with the beginning of lesion formation. From 36h onward, Botrytis growth is linear (Rowe *et al.* 2010) and grow until the entire leaf is consumed. To render a project of this size possible, only the images at 72 hours post infection (hpi) were analyzed. Lesion area at 72h is well defined but hasn't reached large proportion of the leaves. The experiments included three replicates for each isolate x plant genotype in a randomized complete block design and were repeated over two experiments, for a total of six replicates.

Image analysis

The images analysis was performed with an R script as described in (Fordyce *et al.* 2018). Images were transformed into hue/saturation/value (hsv) color space and threshold accounting for leaves color and intensities were defined for each species. Masks marking the leaves and lesions were created by the script and further confirmed manually. The lesions were characterized by counting number of pixel of the original pictures within the area covered by the lesion mask. The numbers of pixels were converted into centimeter using a reference scale within each image.

Data quality control

A dataset of 51,920 lesions was generated in this project but not all leaves inoculated with *Botrytis* developed a visible lesion at 72hpi. These 'failed lesions' can be explained either by technical or biological failures. Technical failures can bias the estimates of the mean. To partition biological and technical failures, the lesion area distribution was analyzed for each species and empirical thresholds

were fixed (Table S5). A lesion below that threshold was considered a technical error only if the median of lesion area for a plant genotype - isolate pair was larger than the threshold. The rationale is the following: when most lesions are of small size, the likelihood of biological reasons for such small lesion areas is high, while when the majority of lesion areas are large, the likelihood of technical error is high. 6,395 lesions (13% of all lesions) were considered as technical failures and removed from the dataset. The statistical analyses and modeling were run on both original and filtered datasets. The removal of technical failures does not impact the effect size of the estimates but their significance.

Statistical analysis

All data handling and statistical analyses were conducted in R (Team 2017). Lesion area was modeled independently for each species using the linear mixed model with lme4 (Bates *et al.* 2014):

Lesion.Area ~ Isolate + Improv/PlantGeno + Isolate*Improv + Isolate*Improv/PlantGeno + (1|Exp.Replicate) + (1|Exp.Tray) + (1|Indiv.Plants)

Where plant genotypes are nested within improvement levels. Experimental replicate and trays as well as the individuals plants on which were collected the leaves for the detached leaf assay are considered as random factors. For each plant genotype, model corrected least-square means (LS-means) of lesion area were calculated for each isolate from with Emmeans with the Satterwaite approximation (Lenth 2018):
Lesion.Area ~ Isolate + (1|Exp.Replicate) + (1|Exp.Tray) + (1|Indiv.Plants)

The meta-analysis model was run over all species LS-means with the linear model:
Lesion.Area ~ Isolate + Species + Species/Improv + Species/PlantGeno + Isolate*Species+ Isolate*Species/Improv + Isolate*Species/PlantGeno

Where improvement levels and plant genotypes where nested within species to account for the phylogenetic common evolutionary history and possibly shared resistance traits of genotypes with low and high levels of improvement.

To visualize relation between isolates and plant genotypes, a heatmap was constructed on standardized LS-means with iheatmapr (Schep & Kummerfeld 2017). The least-square means were standardized (z-score) over each plant genotypes by centering the mean to zero and fixing the standard deviation to one

to overcome the large variation on lesion area across species and large variation in variance linked to the lesion area (Fig 1). Species with low lesion area had also small variance while species with large lesion area presented large variance. Seven isolates that were not consistently infected on all 90 genotypes were dropped, as hierarchical clustering is sensitive to missing data. The unsupervised hierarchical clustering was run with the 'complete' agglomerative algorithm on Euclidean distances. The significance of the dendrogram was estimated with pvclust (Suzuki & Shimodaira 2006) over 20000 bootstraps. The significance of branches was fixed at α =0.95. For the plant genotypes dendrogram, branches were consistently assigned across hierarchical clustering methods (both 'complete' and 'average' algorithms were ran) and bootstrapping while in the *Botrytis* isolates dendrogram, the branches did not show consistency.

The heatmap provides a global picture of how plant genotypes interact specifically with each *Botrytis* isolates. To estimate the global virulence of each isolates, we calculated the mean of the standardized LS-means of lesion area across the eight eudicot species. The host specificity was calculated from the raw LS-means as the coefficient of variation (standard deviation corrected by the mean σ/μ) across the eight species. Low host specificity indicates that isolates grew consistently across the eight species, while high host specificity indicates large variation in lesion area across species (Figure 4B), therefore preference for some species.

Genome-wide association

All isolates were previously whole-genome sequenced at on average 164-fold coverage (Atwell et al. 2018). Host specificity and virulence were mapped using 271,749 SNPs at MAF 0.20 and less than 20% of missing calls with B05.10 genome as reference. The GWA was performed using a generalized ridge regression method for high-dimensional genomic data in bigRR (Shen et al 2013). It imputes a heteroscedastic effect model as effect size estimates rather than p-values. Other GWA methods have been tested for mapping plant-*Botrytis* interactions (Atwell et al. 2018, Corwin et al. 2016, Soltis et al. 2018) and hold comparable results to bigRR model. In particular, Gemma that accounts population structure does not perform significantly better due to the low population structure in the isolate collection (Atwell et al. 2018). Furthermore, BigRR approach was chosen for its known validation rate (Corwin et al. 2016, Fordyce et al. 2018) in the pathosystem. Significance of the effect size was estimated based on 1000 permutations. The 99.9% threshold was used as a conservative threshold for SNP selection although 1000 permutations allow only

an approximation of such a high threshold (Soltis et al., 2018). SNPs were annotated based on their location in ASM83294v1 assembly while gene annotation was extracted from the fungal genomic resources portal (fungidb.org). The Botrytis secretome data including 1220 proteins was extracted from the Fungal Secretome Database (fsd.snu.ac.kr). T4 Protein IDs were converted to B05.10 genome (Simon & Viaud 2018). 896 secreted proteins were successfully identified in B05.10 genomes.

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Data Availability

R codes and datasets will be available as a single archive on Github.

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