Genome-Wide Association Study Identifies Single Nucleotide Polymorphism Markers Associated with Mycelial Growth (at 15, 20, and 25°C), Mefenoxam Resistance, and Mating Type in *Phytophthora infestans*

D. A. Ayala-Usma,^{1,2} G. Danies,³ K. Myers,⁴ M. O. Bond,^{4,5} J. A. Romero-Navarro,⁶ H. S. Judelson,⁷ S. Restrepo,¹ and W. E. Fry^{4,†}

¹ Department of Biological Sciences, Universidad de los Andes, Bogotá, Colombia

² Max Planck Tandem Group in Computational Biology, Universidad de los Andes, Bogotá, Colombia

³ Department of Design, Universidad de los Andes, Bogotá, Colombia

⁴ Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, U.S.A.

⁵ Department of Botany, University of Hawaii, Mānoa, HI, U.S.A.

⁶ Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, U.S.A.

⁷ Department of Microbiology and Plant Pathology, University of California, Riverside, CA, U.S.A.

Accepted for publication 9 December 2019.

ABSTRACT

Phenotypic diversity among individuals defines the potential for evolutionary selection in a species. *Phytophthora infestans* epidemics are generally thought to be favored by moderate to low temperatures, but temperatures in many locations worldwide are expected to rise as a result of global climate change. Thus, we investigated variation among individuals of *P. infestans* for relative growth at different temperatures. Isolates of *P. infestans* came from three collections: (i) individual genotypes recently dominant in the United States, (ii) recently collected individuals from Central Mexico, and (iii) progeny of a recent sexual recombination event in the northeastern United States. In general, these isolates had optimal mycelial growth rates at 15 or 20°C. However, two individuals from Central Mexico grew better at higher temperatures than did most others and two

individuals grew relatively less at higher temperatures than did most others. The isolates were also assessed for mefenoxam sensitivity and mating type. Each collection contained individuals of diverse sensitivities to mefenoxam and individuals of the A1 and A2 mating type. We then searched for genomic regions associated with phenotypic diversity using genotyping-by-sequencing. We found one single nucleotide polymorphism (SNP) associated with variability in mycelial growth at 20°C, two associated with variability in mycelial growth at 25°C, two associated with sensitivity to mefenoxam, and one associated with mating type. Interestingly, the SNPs associated with mefenoxam sensitivity were found in a gene-sparse region, whereas the SNPs associated with growth at the two temperatures and mating type were found both at more gene-dense regions.

The oomycete *Phytophthora infestans*, the causal agent of late blight disease, is considered to be the greatest economic burden for potato growers worldwide (Haverkort et al. 2008). In the United States, late blight is potentially important for nearly all of the approximately 1.1 million acres of potato production (U.S. Department of Agriculture National Agricultural Statistics Service Agricultural Statistics Board). The annual economic impact of potato late blight in the United States has been estimated to be about \$210 million, with the addition of about \$77 million spent on fungicides (Guenther et al. 2001). On tomato, the disease can and has been equally devastating (Fry et al. 2013). In the United States and Canada, pathogen populations have been characterized by waves of migrations and dominance by clonal lineages (Fry et al.

2013). Many of the new clonal lineages are believed to have originated from sexually reproducing populations (Knaus et al. 2016).

Traditionally, isolate characterization within clonal lineages has been studied by means of several phenotypic and genotypic markers. Common phenotypic markers are mating type, virulence spectrum, and metalaxyl resistance (Blandón-Díaz et al. 2012). Genotypic markers include amplified fragment length polymorphism, random fragment length polymorphism fingerprints, and multilocus simple-sequence repeats that, owing to their higher repeatability and resolution, have been selected as the standard genotyping strategy for *P. infestans* (Blandón-Díaz et al. 2012; Cooke and Lees 2004). Diversity studies that consider both phenotypic and genotypic markers have enabled an understanding of population dynamics, which has been useful for disease management (Blandón-Díaz et al. 2012; Cooke et al. 2003; Danies et al. 2013; Day et al. 2004; Hansen et al. 2016; Hermansen et al. 2000; Knapova and Gisi 2002; Petchaboon et al. 2014). It is now possible by next-generation sequencing to perform association tests to find genomic regions that are associated with the phenotype. Of these new technologies, genotyping-by-sequencing (GBS) has allowed researchers to detect and evaluate thousands of genome-wide single nucleotide polymorphism (SNP) markers in clonal populations of *P. infestans* (Hansen et al. 2016), revealing a yet unexplored genetic variation even in the absence of sexual reproduction. However, no published studies to date have exploited GBS to perform associations of genomic variants to phenotypic variability in traits of ecological relevance for the pathogen.

†Corresponding author: W. E. Fry; wef1@cornell.edu

Funding: This work was supported by the Agriculture and Food Research Initiative Competitive Grants Program (grant 2011-68004-30154) from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture, the USDA Sustainable Agriculture Research and Education Program (grant SARE GNE13-056), and the Cornell University College of Agriculture and Life Sciences. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

D. A. Ayala-Usma and G. Danies contributed equally to this work.

*The e-Xtra logo stands for “electronic extra” and indicates that three supplementary figures and three supplementary tables are published online.

The author(s) declare no conflict of interest.

A potentially important phenotypic response is the ability of the pathogen to grow in increasingly warmer temperatures as a consequence of climate change. Such an ability is determined by the phenotypic plasticity of individuals as well as the genetic variation in the population. In a scenario of climate change, daily maxima and minima temperatures are expected to increase by at least 2.8°C in the contiguous United States (Vose et al. 2017) by midcentury. Thus, positively selected genetic variation may result in a durable adaptation of organisms to higher temperature.

An immediately relevant trait in the epidemiology of *P. infestans* is the sensitivity of isolates to fungicides (Goodwin et al. 1996; Saville et al. 2015). For example, the fungicide metalaxyl (now mefenoxam, which is the active *R*-enantiomer) was used with great success in the 1970s and 1980s. However, its widespread application led to the selection of resistance in populations of *P. infestans* in Europe (Davidse et al. 1981; Dowley and O'Sullivan 1981), North America (Goodwin et al. 1996), and the rest of the world since the early 1980s, causing devastating losses (Davidse 1985; Fry and Goodwin 1997; Goodwin et al. 1996). Despite the fact that more than 40 years have passed since the development and commercialization of the molecule, the biological causes of mefenoxam resistance in *P. infestans* have not yet been identified. Randall et al. (2014) postulated that a SNP denominated T1145A, localized in the RPA190 gene, was responsible for a decrease in sensitivity to mefenoxam. This gene was selected for variant screening in a population among other candidates after a series of assumptions about the expected effect of mefenoxam on the ribosomal RNA synthesis (Randall et al. 2014). However, it was later shown through genetic experiments that this variant might not be the causal mutation owing to the lack of cosegregation of the gene with the resistance in the tested strains (Matson et al. 2015). A 2018 work found signals of positive selection in point mutations on the RPA190 gene that might contribute to mefenoxam resistance, but experimental evidence that supports causality was not provided (Chen et al. 2018), thus leaving this basis of insensitivity unresolved.

Since phenotypic variation is the basis upon which selection pressures act to keep or remove genetic variation in populations, it is important to characterize phenotypic diversity to predict the change potential of pathogen populations when facing environmental challenges as a result of artificial or natural selection. Thus, our aim was to assess the phenotypic diversity for mycelial growth at warm temperature and for mefenoxam sensitivity in a panel of *P. infestans* isolates to investigate diversity on which different selection pressures, such as a warming climate and fungicide use, might act. Mating type (A1 or A2) was included as a trait that is presumably under simple genetic control. To date, this is the first GBS analysis performed to identify genomic regions associated with such diversity and to identify genes potentially responsible for such phenotypic variation in *P. infestans*. It was possible to recover six SNPs associated with the evaluated phenotypes.

MATERIALS AND METHODS

Isolates and phenotyping. *Isolates.* The panel of isolates included three groups. Group i was composed of six U.S. clonal lineages (US-7, US-8, US-11, US-22, US-23, and US-24) whose genetic and phenotypic profile has already been investigated. Group ii was composed of 16 isolates that have characteristics of a progeny from a sexually reproducing population collected in and around west-central New York State in 2010 and 2011 (referred to hereafter as the NYS-2010/11 or GDT population) (Danieš et al. 2014). Group iii was composed of 31 isolates collected in Central Mexico where sexual reproduction is common. For one of the six U.S. clonal lineages (US-23), six individuals that showed differences in their microsatellite profiles were included. Isolates were maintained on pea agar (Jaime-Garcia et al. 2000) containing ampicillin (100 µg ml⁻¹),

rifampicin (125 µg ml⁻¹), and pentachloronitrobenzene (25 µg ml⁻¹) at 20°C.

Effect of temperature on mycelial growth (dry weight). To determine the effect of temperature on mycelial growth, 1-cm-diameter discs of hyphae growing on agar medium of each lineage were placed in a 100 × 15-mm Petri plate containing 10 ml of vacuum-filtered pea broth. Each replicate consisted of four plates, each incubated at one of four different temperatures (10, 15, 20, and 25°C) for 8 days. Mycelia were subsequently dried using vacuum filtration, frozen at -80°C, lyophilized, and weighed. There were at least two replicates of each isolate.

Effects of temperature and lineage on mycelial growth were analyzed using a mixed effects model analysis with the *lmerTest* R package (Kuznetsova et al. 2017), where temperature, lineage, and their interaction were fixed effects and replicate nested within lineage was considered a random effect. Differences in square-root-transformed dry weight measurements among lineages as a function of temperature at 10, 20, and 25°C normalized relative to 15°C were identified using pairwise least-squares means comparison based on the mixed model and applying Tukey's *P* value adjustment as implemented in the *lsmeans* R package (Lenth 2016).

Mefenoxam sensitivity. Mefenoxam sensitivity of isolates was assessed as described previously by Therrien et al. (1993), except that mefenoxam was used in place of metalaxyl. Isolates were grown on pea agar amended with Ridomil Gold SL (Syngenta, Greensboro, NC) such that the final concentrations of the active ingredient (mefenoxam) were 0, 5, or 100 µg ml⁻¹. Mycelial plugs (8 mm in diameter) were obtained from actively growing cultures, transferred to the test plates, and incubated for approximately 10 to 12 days or until growth on the control plate (0 µg ml⁻¹) was approximately 75 to 90% of the diameter of the Petri plate. Assessment of mefenoxam sensitivity was determined on the basis of radial growth of cultures grown on plates amended with mefenoxam (5 and 100 µg ml⁻¹) compared with nonamended controls. Growth at 5 and 100 µg ml⁻¹ was represented as a proportion of the growth on the nonamended control plates.

Mating type. For all isolates, mating type was determined by pairing an unknown isolate with an isolate of known mating type on an agar culture plate and observing for the production of oospores. The A1 tester strain was US970001 (US-17 genotype) and the A2 tester was US040009 (US-8 genotype). Matings were assayed on rye B (Caten and Jinks 1968) or pea agar (Jaime-Garcia et al. 2000) media with β-sitosterol. Saturated β-sitosterol solution was prepared by adding approximately 1 g of solid β-sitosterol to approximately 30 ml of 100% ethanol and then vortexing for at least 1 min. We subsequently allowed the β-sitosterol that did not go into solution to settle, and we used the supernatant (saturated solution of β-sitosterol) for Rye B or pea medium (1 ml of saturated β-sitosterol per 1 liter of medium). Petri plates were kept at 20°C for 10 to 14 days. The hyphal interface of the two colonies was investigated microscopically using 125× magnification. Isolates that formed oospores at the interface with the known A1 isolate were designated A2 and those that formed oospores with the known A2 isolate were designated A1. The known isolates (A1 and A2) were paired as positive controls, whereas negative controls paired the A1 or A2 testers with themselves.

Isolation of single zoospores was performed for isolates that produced oospores in the presence of both the A1 and the A2 mating type testers as well as in the negative control (unknown isolate paired with itself). To do this, sporangia were washed with sterile distilled water from sporulating lesions on leaflets. The sporangial suspensions were adjusted to 8,000 sporangia per ml using a hemocytometer and maintained at 4°C for 3 to 4 h to induce zoospore release. Aliquots of the zoospore suspension were subsequently spread on 100-mm Petri plates containing 20 ml of water agar (0.75%). Three different aliquots (20, 40, or 100 µl) were used with one aliquot spread on each plate. The zoospore suspension was spread using a sterile glass rod. Plates were then

kept at 10°C for 12 h to encourage zoospore germination and subsequently maintained at 15°C for another 24 h. Individual germinated zoospores were then picked using a sterile scalpel and placed onto pea agar medium. Colonies formed from single zoospores were again tested for mating type as explained above. The extended results of this experiment can be observed in Supplementary Table S1.

Genotyping, population structure, and linkage disequilibrium analyses. *Assembly and annotation of the P. infestans 1306 draft genome.* A draft genome assembly of *P. infestans* strain 1306 was generated from 100-fold coverage of long reads obtained using a Pacific Biosciences RSII instrument and from 66-fold coverage of 350-nt paired-end reads generated using an Illumina MiSeq system. Reads were assembled and error-corrected using FALCON and Quiver (Chin et al. 2016) using `length_cutoff` and `length_cutoff_pr` parameters of 10000 and 14800, respectively (*data not shown*, available upon request). This reference genome was used because of better assembly statistics compared with the available RefSeq genome (Supplementary Table S2). Genome annotation was performed by using a combination of ab initio predictions, homology scans against RefSeq protein and mRNA sequences of the *P. infestans* T30-4 genome, and repetitive element detection as implemented in MAKER pipeline version 2.31.9 (Holt and Yandell 2011). The predicted protein dataset annotation was refined by BLAST searches against the UniRef90 database and by protein domain identification using InterProScan version 5.25-64.0 (Jones et al. 2014).

GBS of the phenotyped isolates. Using genomic DNA isolated with a DNeasy Plant Mini Kit (QIAGEN), GBS was performed as described by Elshire et al. (2011) at the Cornell University Institute of Genomic Diversity. Briefly, genome complexity was reduced by digesting total genomic DNA from individual samples with the type II restriction endonuclease *ApeKI*, which recognizes a degenerate 5-bp sequence (GCWGC, where W is A or T) and creates a 5' overhang (3 bp). Digested products were then ligated to Illumina adapters with enzyme-compatible overhangs, containing the barcode sequence and a binding site for the Illumina sequencing primers. Samples were then pooled, purified, and amplified with primers compatible to the adapter sequences. GBS library fragment-size distributions were checked on a BioAnalyzer (Agilent Technologies Inc.). The PCR products were quantified and diluted for sequencing on the Illumina HiSeq 2500 system.

Most samples were sequenced in triplicate ($n = 47$), whereas four US-23 isolates, five Mexican isolates, and two NYS-2010/11 isolates were sequenced in duplicate. All of these served as technical replicates. A total of 165 samples (including two DNA-free controls) were multiplexed in four Illumina flow cell lanes. The NGSEP version 3.0.2 GBS pipeline (Duitama et al. 2014) was used for SNP discovery and annotation. Sequence reads were deconvoluted, trimmed for adapters, and mapped against the draft genome of *P. infestans* strain 1306. After variant calling and merging of technical replicates, SNPs presenting a minor allele frequency <0.05 , a genotyping quality score ≤ 40 , a minimum distance between variants ≤ 20 , and presence of each genotype in $<80\%$ of the individuals were filtered out.

Inference of population structure. Three replicate runs of STRUCTURE version 2.3.4 (Hubisz et al. 2009) were performed for values of K (putative genetic groups) ranging from 1 to 8. For each analysis, the numbers of burn-in iterations and MCMC repetitions were set to 10,000 and 100,000, respectively. The most likely K was determined by the delta-K statistic, as implemented in STRUCTURE HARVESTER version 0.6.94 (Earl and vonHoldt 2012). The resulting population membership probability matrix (Q-matrix) was subsequently used in the association tests to correct for population stratification in the association analysis (see below).

Linkage disequilibrium decay. Linkage disequilibrium (LD) decay was calculated to identify genomic regions that cosegregate with associated SNP markers. All of the pairwise r^2 correlation

coefficients for the recovered SNPs were calculated using PLINK version 1.9 (Chang et al. 2015). Coefficients from SNP pairs in the same genomic scaffold were plotted against their physical distance and a smoothing spline fitting for such data was performed in R version 3.4.1 (R Core Team 2018). The LD threshold was estimated as the distance that corresponds to the 95th percentile of all of the ordered pairwise r^2 correlation coefficients among the SNPs that were found in different genomic scaffolds as described in Breseghello and Sorrells (2006).

Genome-wide association for quantitative and binary traits. To find genetic markers related to the variation in mycelial growth at 10, 20, and 25°C and to variation in sensitivity to mefenoxam, association tests were performed using the best linear unbiased predictors (BLUPs) of the collected phenotypic data for a total amount of 58 isolates in the entire panel. To account for possible effects of the non-normal distribution of the BLUPs, a Box-Cox transformation of the data was applied, as implemented in the geoR package (Ribeiro and Diggle 2001) for R version 3.4.1. Mixed linear models (MLMs) for the associations were fitted in TASSEL version 5 (Bradbury et al. 2007) using the STRUCTURE Q-matrix obtained above and an identity-by-state kinship matrix (K) to control for cryptic relatedness between the samples. To establish the significance of the associated SNPs, false discovery rate thresholds of 5 and 10% were calculated with the Holm-Bonferroni method (Holm 1979). All SNPs that fell below the threshold were considered as significant for the measured trait. The genome-wide association for mating type, a binary trait, was performed using a logistic MLM as implemented in the GMMAT R package (Chen et al. 2016) with the previously obtained K-matrix as a covariate to control for relatedness effects, and the significance assessment was identical to the one used for quantitative traits.

To explore the genomic context in which the significant SNPs occur, a window of 14,141 bases (corresponding to a region in LD, as determined above) downstream and upstream of the significant SNPs was recovered. For the genes found within these regions, the GO, InterPro, Pfam, and SUPERFAMILY annotation terms were analyzed. Repetitive elements within this region, as well as unidentified hypothetical genes, were also considered for the analysis.

RESULTS

Isolates and phenotyping. *Effect of temperature on mycelial growth (dry weight).* The effect of temperature on mycelial dry weight was assessed for 56 of the 58 isolates (Fig. 1). There were 12 isolates for which temperature did not have a significant effect on growth (Fig. 1). For all of the remaining isolates, growth at 10°C was slower than at 20 or 25°C. Isolates MX-15, MX-21, and MX-34 showed significantly better growth at 20°C than at 25°C. Two isolates, MX-05 and MX-18, grew best and equally well at both 20 and 25°C, whereas MX-13 and MX-15 grew relatively more slowly at both 20 and 25°C (Figs. 1 and 2). When statistical differences were visualized as paired comparisons among isolates, those that had been collected in the United States were not different from each other in terms of growth response to temperature (Fig. 2).

Mefenoxam sensitivity. Sensitivity to mefenoxam was assessed for 54 of the 58 isolates. In the U.S. group, isolates belonging to clonal lineages US-7 and US-11 were highly resistant (showing a growth proportion of $>40\%$ relative to the control at both 5 and 100 $\mu\text{g ml}^{-1}$ of mefenoxam); the isolate belonging to clonal lineage US-8 displayed an intermediate resistance (growth of $>40\%$ only at 5 $\mu\text{g ml}^{-1}$), and isolates belonging to clonal lineages US-22, US-23, and US-24 were generally sensitive to mefenoxam (Fig. 3A). The NYS-2010/11 isolates were mostly sensitive to mefenoxam, with GDT-09 being the single intermediate resistant isolate. In contrast, isolates from Mexico showed a wider variety of responses to mefenoxam (Fig. 3B). Seven isolates were resistant to mefenoxam, nine displayed an intermediate phenotype, and 15 were sensitive.

For two isolates (MX-18 and MX-27), mycelial growth seemed to be enhanced by the presence of mefenoxam.

Mating type of the isolates in the panel. In total, there were 31 isolates of the A1 mating type, 20 isolates of the A2 mating type, and six isolates that were self-fertile (they produced oospores with either an A1 tester or an A2 tester, or in pure culture) (Supplementary Table S1). Self-fertile isolates retained this trait in single-zoospore cultures. Isolate MX-33 was lost before mating type was determined.

Genotyping, population structure, and LD analyses.

Reference genome and variants recovered with GBS. To associate genomic regions with phenotypic traits, we conducted a genome-wide association study (GWAS) using the draft genome of *P. infestans* strain 1306 as the reference sequence. The strain 1306 genome was chosen given its overall better assembly statistics compared with the current RefSeq genome of the species (Haas et al. 2009). A greater assembly size and better contiguity of the genome (Supplementary Table S2) allowed the possibility of accessing a larger number of positions within the genome that would otherwise be inaccessible with the current RefSeq assembly. For this assembly, we were able to predict 27,917 coding elements that were used as the basis for subsequent analyses.

The total number of reads obtained by the GBS method for the sequenced panel was 559,421,723. Of the total number of reads,

26.9% were aligned to unique positions, 67.3% were aligned to multiple positions (as expected for a genome with such repetitive content), and 5.7% could not be aligned to the reference genome. This represents an overall 94.2% alignment rate. After merging the technical replicates and performing raw variant calling, we recovered 854,002 variants for the dataset with an average density of 2.94 SNPs per kb and an average fold-coverage of 3.32× per SNP. The SNP filtering strategy used in this study aimed to obtain only those variants that are genotyped in the majority of the sampled individuals at an allele frequency high enough to perform a balanced statistical association. The applied filters reduced the total number of variants to 11,175 high-quality SNPs with an average density of 0.08 SNPs per kb.

Population structure. We assessed the population structure of the isolates to be certain that it was appropriate for GWAS analysis. The STRUCTURE inference of population stratification subdivided the isolates into six genetic groups (Fig. 4). Among these, three clearly defined clonally reproducing genetic groups were observed. These three groups were composed of the following: (i) isolates belonging to the NYS-2010/11 grouping and US-22 (yellow bars in Fig. 4), (ii) isolates belonging to the US-23 clonal lineage (dark green bars in Fig. 4), and (iii) Mexican isolates MX-22, MX-30, and MX-33 (purple bars in Fig. 4). Isolates US-8 and US-24 presented a high degree of admixture similar to the majority

Fig. 1. Mycelial growth (dry weight) of **A**, U.S. and **B**, Mexican *Phytophthora infestans* isolates measured at 10, 20, and 25°C (red, green, and blue bars, respectively) relative to growth at 15°C (dashed line). Bar height represents the median value of the replicates and the error bars represent the median absolute deviation. Statistical analyses were performed on square root-transformed data. Significant differences among treatments for an isolate are shown with the squares, triangles, and diamonds at the top of each panel.

of Mexican strains to which they are closely related. Lineages US-7 and US-11 clustered together with MX-10, MX-27, and MX-35, which seem to share a genotype more closely related to the Mexican isolates than to any of the other U.S. isolates studied (Fig. 4). The analysis reveals that population stratification exists in the panel;

thus, the population structure matrix obtained was used to account for the effects that such stratification might have in the following association tests.

Six SNPs are significantly associated with variability in the measured traits. Tests of the statistical association between SNPs

Fig. 2. Pairwise least-squares means comparisons among all of the *Phytophthora infestans* isolates in the panel for mycelial growth (dry weight) at 20 and 25°C normalized to 15°C. All statistical analyses were performed on the square-root transformations of the raw data. A white square indicates that there is no significant difference in the temperature response of the isolates being compared. A green square indicates that there is a significant difference for relative growth at 20°C; an orange square indicates a significant difference for growth at 25°C; and a blue square indicates a significant difference for relative growth at both 20 and 25°C. The arrows indicate the “direction” of the difference; for example, if the arrow is pointing upward, then the isolate in the column has a higher relative growth rate than the isolate in the row. Whenever differences at both temperatures are observed, two arrows are drawn to indicate the shift at each temperature. For example, isolate MX-05 grows relatively faster at 25°C than do most GDT isolates, and most MX isolates grow relatively slower at 25°C than does MX-05. Thus, MX-05 appears to tolerate higher temperatures more readily than do most other isolates.

and phenotypes was performed by fitting MLMs with Box-Cox transformed BLUPs of the phenotypes as inputs (Supplementary Figs. S1 and S2) for a total of 58 isolates comprising the entire panel. This was done to correct as much as possible for distribution skewness. Correction for population structure and cryptic relatedness was made using Q and K matrices as secondary inputs, respectively. For the binary mating type phenotype, only the K-matrix was used. The obtained *P* value distributions of the association tests performed for each phenotype were approximately uniform as observed in the Q-Q plots shown in Figure 5. This indicates that effects of population stratification are effectively accounted for in our models. Upon drawing corrected significance thresholds, several SNPs were associated with phenotypes. One SNP was associated with the variability in growth at 10°C (Fig. 5A), one SNP for variability in growth at 20°C (Fig. 5B), and one for variability in growth at 25°C (Fig. 5C). Two SNPs were associated with variability in sensitivity to mefenoxam at 100 µg ml⁻¹ (Fig. 5D). Finally, one SNP was associated with variability in mating type (Fig. 5E).

Given our low final SNP marker density (0.08 SNPs per kb) relative to the whole genome size, we expect the significantly associated positions to be a proxy of other putative causal positions

that are in LD with them. To analyze the genomic context of the significantly associated SNPs, we calculated the maximum physical distance in our assembly that could be considered in LD with a given SNP. It was observed that the LD decays to a level indistinguishable from noise at 14,000 bases away from the SNP (*r*² = 0.15) (Supplementary Fig. S3). Thus, we established a window of 14 kb upstream and 14 kb downstream of the statistically significant SNP that would contain all of the genomic elements that will probably segregate along with the genotyped marker. All of the details of the genomic elements and functions recovered for the LD window for each SNP can be examined in Supplementary Table S3.

Variability in temperature responses is associated with transposon activity, solute trafficking, and water balance. There was a single associated SNP for variability at each evaluated growth temperature. The SNP associated with growth at 10°C (*P* = 1.4×10^{-4}) was located in the position 516952 of contig 000028F (abbreviated as 000028F:516952). There were 10 genomic elements within the LD window: six were long terminal repeat (LTR) retrotransposons (five Copia and one Gypsy), three were genes with unknown function, and one was a gene that contained a hAT transposase dimerization domain (InterPro term: IPR008906) and a ribonuclease H domain (IPR012337) (Fig. 6A). The SNP

Fig. 3. Response of isolates of *Phytophthora infestans* to mefenoxam. **A**, Isolates belonging to six U.S. clonal lineages and 16 isolates that seem to have characteristics of a sexually reproducing population collected in and around west-central New York State in 2010 and 2011 (GDT lineages). **B**, Thirty isolates collected in Central Mexico where sexual reproduction is common. Relative growth as a percentage of control (0 µg ml⁻¹), at 5 µg ml⁻¹ (pink bars), and 100 µg ml⁻¹ (blue bars). Bars represent the median value and error bars represent the median absolute deviation. The dashed line represents a growth of 40% relative to control used as a resistance threshold.

associated with diversity for growth at 20°C ($P = 6.13 \times 10^{-6}$) was 000002F:1096811. There were 11 genomic elements within the examined LD window: five LTR retrotransposons (four Copia and one Gypsy), two genes that encoded proteins with Aquaporin (IPR023271) and Major Intrinsic Proteins (IPR000425)-related terms, one gene belonging to a Clavamine synthase-like superfamily (SSF51197), and three genes whose function could

not be assigned (Fig. 6B). Finally, the SNP associated with diversity for growth at 25°C ($P = 5.3 \times 10^{-5}$) was SNP 000141F:215593, which is located in a gene-rich region. One DNA transposon of the Mariner genus was found in the region (Fig. 6C). The 11 genes in the LD window had very diverse annotated functions, including transcriptional regulation (transcription initiation factor TAFII31 [IPR003162]; BRCT domain [IPR001357]; RNA-polymerase II-associated protein

Fig. 4. Population stratification estimated by STRUCTURE from a set of 11,175 high-quality single nucleotide polymorphisms surveyed for U.S. and Mexican clonal lineages of *Phytophthora infestans*. The colored bars depict membership probabilities of the clonal lineages to six putative subpopulations of *P. infestans* as inferred by STRUCTURE HARVESTER.

3-like, C-terminal domain [IPR025986]; and WD40-repeat-containing domain [IPR017986]); cell structure and remodeling functions (Formin, FH2 domain [IPR015425]; and Hemingway/KIAA1430 [IPR029488]); and solute trafficking and transport (Polycystin cation channel, PKD1/PKD2 [IPR013122]; amino acid exporter protein, LeuE-type [IPR001123]; C2 domain [IPR000008], mitochondrial substrate/solute carrier [IPR018108], and folate-biopterin transporter [IPR004324]). For further details on the functions associated with each gene found, see Supplementary Table S3.

Transposon-rich regions are associated with the phenotypic response to mefenoxam. The first SNP associated with diversity to mefenoxam sensitivity ($P = 6.8 \times 10^{-7}$) was found in the contig 000028F at the position 602284 (000028F:602284). There were 15 genomic elements within the LD window analyzed. This SNP was found to be in a very gene-sparse region rich in transposons and repeats. There were eight retrotransposons, of which five were LTR elements of the Gypsy family and three were long interspersed nuclear element retrotransposons. Two DNA transposons were identified, one from the PiggyBac class and another from the Crypton class (Fig. 6D). Two short tandem repeats of 24 and 50 bp, respectively, were also identified as neighboring genomic elements of the SNP. Of the three genes recovered, a putative function could only be assigned to one, which

corresponded to a RXLR phytopathogen effector protein (IPR031825). The second SNP associated with diversity in mefenoxam sensitivity ($P = 1.2 \times 10^{-4}$) was 000018F:625396 (Fig. 6E). Again, three genes were recovered in the window, but only one had a putative function corresponding to a DDE superfamily endonuclease (PF13358), which is a transposon endonuclease. The repetitive content of this region included one LTR retrotransposon of the Gypsy family, one Crypton DNA transposon, and two Helitron DNA transposons.

Mating type is associated with a gene-dense region containing signaling, metabolic, and structural functions. SNP 000108F:417461 ($P = 3.4 \times 10^{-4}$) was found to be significantly associated with the mating type trait. There were 10 genes in the LD window around this SNP (Fig. 6F). The annotations of these genes had functional terms related to cell signaling (serine-threonine/tyrosine-protein kinase, catalytic domain [IPR001245]), metabolic processes (Orn/DAP/Arg decarboxylase 2, N-terminal [IPR022644]), and cell structure (Actin family [IPR004000]). Two genes encoding different types of DNA binding proteins were also in this region, which contained Mg-dependent DNase, TatD type (PIRSF005902), and Myb/SANT-like DNA-binding domains (PF12776), respectively. A short tandem repeat of the thymine (T) was also present in this region.

Fig. 5. Manhattan plots and QQ-plots of the performed genome-wide association (GWA) tests for 11,175 single nucleotide polymorphisms. **A, B, and C,** Mycelial growth (dry weight) at 10, 20, and 25°C, respectively, relative to 15°C. **D,** Sensitivity to mefenoxam 100 µg mL⁻¹. **E,** mating type. The statistical association models used were mixed linear models with structure (Q-matrix) and kinship (K-matrix) correction as implemented in TASSEL for quantitative traits (A to D) and a logistic mixed model with K-matrix correction for the binary mating type trait (E), as implemented in the GMMAT package. Significance thresholds were the Bonferroni correction with alpha = 0.05 and false discovery rate (FDR) at 5 and 10%. Lambda (λ) is the genomic inflation factor of the GWA.

DISCUSSION

In this study, we investigated the phenotypic diversity of a panel of 58 *P. infestans* isolates from the United States and Mexico for three main agriculturally relevant traits: mycelial growth at increasingly warmer temperatures, sensitivity to mefenoxam, and mating type. We performed genome-wide association tests for each of the phenotypes using genomic variants recovered by GBS to gain insights into the genomic basis of these traits. It is important to consider that sample size has an effect on the association discovery rate. Phenotyping traits with high accuracy and precision is both costly and time-consuming, thus limiting the number of isolates that can be included. However, *P. infestans* allows replication of individual genotypes both within and across multiple environments, allowing for far more precise phenotyping because the amount of environmentally induced variation can be estimated and partitioned out in association analyses (Ingvarsson and Street 2011). This is the first study to date that attempts to find significant associations for these phenotypes at a genomic scale in *P. infestans*.

Effect of temperature on mycelial (dry weight). Our data show that, in general, isolates grew better at 15 or 20°C than at 10°C.

Whenever differences could be evidenced among 20 and 25°C, the former showed to be the optimal temperature as reported previously in literature (Mariette et al. 2016; Mizubuti and Fry 1998; Sujkowski 1987). Data presented in Mariette et al. (2016) suggest that sporangia production in planta has a maximum around 18°C but it is hindered at higher temperatures (such as 24°C) despite the fact that lesion growth rate seems to be unaffected by warmer temperatures.

We found three loci to be statistically associated with variability in mycelial growth (dry weight) at several temperatures. The association of the transposon-rich locus (SNP 000028F:516952) raises the question of whether temperature responses in *P. infestans* populations might be related to transposon activity as result of adverse conditions or thermal adaptation. Previous studies have shown that a relationship between thermal stress and transposon activation for certain elements of the Tc1-Mariner family in *Aspergillus oryzae* (Ogasawara et al. 2009) and LTR-Copia elements in *Vigna angularis* (Masuta et al. 2018) and *Arabidopsis thaliana* (Cavrak et al. 2014) exists. Such behavior has been postulated to be a product of a transient release of the epigenetic regulation of certain heterochromatic regions (Pecinka et al. 2010).

Fig. 6. Genomic context analysis of the single nucleotide polymorphisms (SNPs) associated with the phenotypic data. The genomic elements for which the annotation was recovered were those found within a 14-kb linkage disequilibrium window up- and downstream of the associated variant. **A, B, and C**, SNPs associated with optimal mycelial growth at 10, 20, and 25°C, respectively. **D and E**, SNPs associated with sensitivity to mefenoxam at 100 µg ml⁻¹. **F**, SNP associated with mating type.

Given that the associated SNPs found in this study fall near *TeMariner* and *LTR-Copia* elements, there is also the possibility that they point to mutations that affect the mobility of such elements. This remains to be explored in further experimental work.

The SNP 000002F:1096811 was found in a region with a greater content of genes. The enrichment of solute and water transport-associated terms might indicate some degree of variation within the *P. infestans* population in terms of hydric dynamics of the organism involved in hyphal growth. Aquaporins have been observed to play a role in temperature-related phenomena such as freezing tolerance in *Saccharomyces cerevisiae* and *Candida albicans* (Tanghe et al. 2002, 2005).

The SNP 000141F:215593 was found in a gene-rich region with 12 putative genes containing functional terms related to cell signaling and gene expression processes, small molecule transport such as ions and amino acids, and cytoskeleton-related functions. The presence of polycystin cation channel (IPR013122), C2 (IPR000008), and Pleckstrin (PH-like, IPR011993) domains near the recovered marker suggests a potential association between temperature and calcium signaling. Silverman-Gavrila and Lew (2001, 2002, 2003) showed that hyphal growth in the fungus *Neurospora crassa* relies on a tip-high Ca^{2+} cytosolic concentration established through the activity of inositol-1,4,5-trisphosphate-activated Ca^{2+} channels. Ca^{2+} ions can be mobilized from intracellular sources through transient receptor potential channels, such as polycystin, via the phospholipase C (PLC) pathway and can be modulated by PLC activity (Gonçalves et al. 2014). The presence of C2 (a calcium/lipid binding domain) and PH-like domains (which bind to lipids) in the PINF_1306_0020389 gene might suggest its role as a phospholipase (Kadamur and Ross 2016). Functional studies in the role of PLC genes from *C. albicans* and *S. cerevisiae* showed that mutants for PLC in both species exhibited temperature sensitivity, osmosensitivity, and reduced growth in media with carbon sources other than glucose (Flick and Thorner 1993; Kunze et al. 2005).

Mefenoxam sensitivity. Differences in sensitivity to mefenoxam were observed among the isolates of *P. infestans* assessed. Highly sensitive and highly resistant isolates were identified in both the Mexican and the U.S. populations. The congruence between the phenotypic response to mefenoxam measured in this study with previously reported data for strains representative of clonal lineages from the United States (Danes et al. 2013) served as a validation for our new data: older lineages (US-7, US-8, and US-11) were intermediate to highly resistant and more recent lineages (US-22, US-23, and US-24) were sensitive to the fungicide. Isolates from the NYS-2010/11 (GDT) population were mostly sensitive to mefenoxam. However, despite their highly uniform genetic background, some variability in sensitivity was observed among this population with one individual (GDT-09) displaying intermediate resistance to the fungicide. As expected, isolates belonging to the sexually reproducing Mexican population were highly diverse in their response to mefenoxam.

Two loci were statistically associated with phenotypic variation in mefenoxam sensitivity. Upon examination of the genomic elements that fall within the calculated LD window around the SNP, it was evident that such loci are gene-sparse compartments of the genome that contained a large number of transposon-related sequences. It has been observed in fungi that continuous exposure to fungicides in *Monilinia fruticola* induces transposon movement, thus generating novel mutations (Chen et al. 2015). There are two main possibilities for the role of transposable elements in the phenotypic variability of mefenoxam sensitivity. The first is that the marker accounts for a mutation that alters the activity of transposable elements, which might further affect loci involved in fungicide sensitivity. The second possibility is that the marker indicates a change in a region that affects the expression of fungicide sensitivity-related genes such as a promoter or an enhancer region. Whatever the case is, it remains to be investigated.

In previous genetic diversity studies quantitative loci (MEX loci), identified by random amplification of polymorphic DNA, were postulated to be associated with mefenoxam resistance (Judelson and Roberts 1999). These loci were identified by amplification of genomic regions using W4 and AN18 primers (Judelson and Roberts 1999). After an in silico search of these regions in the genome of *P. infestans*, we did not find any overlap between any putative MEX locus and our mefenoxam-associated loci. Thus, no evidence for the involvement of the MEX loci in our study was found. Moreover, we did not find coding elements with functions similar to RPA190 in our study. These findings are expected from a complex trait such as fungicide sensitivity that might involve multiple loci, and we cannot rule out the possibility of involvement of other loci such as MEX or RPA190 in this phenotype (Judelson and Roberts 1999; Randall et al. 2014).

Mating type. Among the panel of isolates assessed, we identified individuals of the A1 and the A2 mating type. Interestingly, six of the 36 Mexican isolates assayed seemed to be “self-fertile.” A single SNP marker associated with the binary mating type phenotype in the evaluated panel was identified. Among the genes found in LD with the marker, one (PINF_1306_0018565, located in contig 000108F:416235-417636) containing a Myb/SANT-like DNA-binding domain (PF12776) was found. This domain is also present in the Rtf1 protein of *Schizosaccharomyces pombe* that binds to a region named replication termination site 1 (RTS1) near the mating-type locus 1 (Eydmann et al. 2008). The molecular interaction of Rtf1 with RTS1 in *S. pombe* blocks the progression of the replisome complex at the moment of DNA synthesis in a strand-specific direction, allowing only one replisome to move through the mat1 locus and to generate an epigenetic imprint mark that will lead to a recombination-mediated mating-type switching that is coupled with the replication process (Gadaleta and Noguchi 2017). Given the previous study, our new evidence, and the lack of knowledge on the mechanisms that determine mating type in *P. infestans*, this gene could be further investigated to understand its involvement in the sexual reproduction of this organism.

Conclusions. This is the first study that identifies markers associated with specific phenotypic traits in *P. infestans* using GBS. These markers are needed to enable predictions of phenotype from analyses of genomes and to develop an accurate understanding of the genetic basis of phenotypic traits. Current population sizes used in association mapping studies are modest in size and need to be greatly increased if mutations explaining less than a few percent of the phenotypic variation, or rare mutations with very low minor allele frequency in the populations, are to be detected (Ingvarsson and Street 2011). Phenotypes are important to disease management. If a phenotype is detected rapidly, management efforts can be targeted to the specific characteristics of the pathogen. In very simple pathogen populations, once the phenotypes of clonal lineages are defined it is then feasible to use the clonal lineage assignment of a new isolate as a surrogate for phenotype (Small et al. 2015). However, it is time prohibitive to characterize each lineage in a complex (e.g., sexual) population, in which case genetic markers associated with a specific phenotype are important. Thus, an ultimate goal is to develop a DNA-based assay that would allow the identification of phenotypic traits of interest.

LITERATURE CITED

- Blandón-Díaz, J. U., Widmark, A.-K., Hannukkala, A., Andersson, B., Högborg, N., and Yuen, J. E. 2012. Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. *Phytopathology* 102:323-330.
- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., and Buckler, E. S. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 23:2633-2635.
- Breseghele, F., and Sorrells, M. E. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics* 172: 1165-1177.

- Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
- Cavrak, V. V., Lettner, N., Jamge, S., Kosarewicz, A., Bayer, L. M., and Mittelsten Scheid, O. 2014. How a retrotransposon exploits the plant's heat stress response for its activation. PLoS Genet. 10:e1004115.
- Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., and Lee, J. J. 2015. Second-generation PLINK: Rising to the challenge of larger and richer datasets. Gigascience 4:7.
- Chen, F., Everhart, S. E., Bryson, P. K., Luo, C., Song, X., Liu, X., et al. 2015. Fungicide-induced transposon movement in *Monilinia fructicola*. Fungal Genet. Biol. 85:38-44.
- Chen, F., Zhou, Q., Xi, J., Li, D., Schnabel, G., and Zhan, J. 2018. Analysis of *RPA190* revealed multiple positively selected mutations associated with metalaxyl resistance in *Phytophthora infestans*. Pest Manag. Sci. 74:1916-1924.
- Chen, H., Wang, C., Conomos, M. P., Stilp, A. M., Li, Z., Sofer, T., et al. 2016. Control for population structure and relatedness for binary traits in genetic association studies via logistic mixed models. Am. J. Hum. Genet. 98: 653-666.
- Chin, C. S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., et al. 2016. Phased diploid genome assembly with single-molecule real-time sequencing. Nat. Methods 13:1050-1054.
- Cooke, D. E. L., and Lees, A. K. 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. Plant Pathol. 53:692-704.
- Cooke, D. E. L., Young, V., Birch, P. R. J., Toth, R., Gourlay, F., Day, J. P., et al. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). Plant Pathol. 52:181-192.
- Danies, G., Myers, K., Mideros, M. F., Restrepo, S., Martin, F. N., Cooke, D. E. L., et al. 2014. An ephemeral sexual population of *Phytophthora infestans* in the Northeastern United States and Canada. PLoS One 9: e116354.
- Danies, G., Small, I. M., Myers, K., Childers, R., and Fry, W. E. 2013. Phenotypic characterization of recent clonal lineages of *Phytophthora infestans* in the United States. Plant Dis. 97:873-881.
- Davidse, L. C. 1985. Resistance to acylalanines in *Phytophthora infestans* in the Netherlands. EPPO Bull. 15:403-409.
- Davidse, L. C., Looijen, D., Turkensteen, L. J., and van der Wal, D. 1981. Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Neth. J. Plant Pathol. 87:65-68.
- Day, J. P., Wattier, R. A. M., Shaw, D. S., and Shattock, R. C. 2004. Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995-98. Plant Pathol. 53:303-315.
- Dowley, L. J., and O'Sullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 24:417-421.
- Duitama, J., Quintero, J. C., Cruz, D. F., Quintero, C., Hubmann, G., Foulquié-Moreno, M. R., et al. 2014. An integrated framework for discovery and genotyping of genomic variants from high-throughput sequencing experiments. Nucleic Acids Res. 42:e44.
- Earl, D. A., and vonHoldt, B. M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359-361.
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379.
- Eydmann, T., Sommariva, E., Inagawa, T., Mian, S., Klar, A. J. S., and Dalgaard, J. Z. 2008. Rtf1-mediated eukaryotic site-specific replication termination. Genetics 180:27-39.
- Flick, J. S., and Thorne, J. 1993. Genetic and biochemical characterization of a phosphatidylinositol-specific phospholipase C in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 13:5861-5876.
- Fry, W. E., and Goodwin, S. B. 1997. Re-emergence of potato and tomato late blight in the United States. Plant Dis. 81:1349-1357.
- Fry, W. E., McGrath, M. T., Seaman, A., Zitter, T. A., McLeod, A., Danies, G., et al. 2013. The 2009 late blight pandemic in the Eastern United States – Causes and results. Plant Dis. 97:296-306.
- Gadaleta, M., and Noguchi, E. 2017. Regulation of DNA replication through natural impediments in the eukaryotic genome. Genes (Basel) 8:98.
- Gonçalves, A. P., Cordeiro, J. M., Monteiro, J., Muñoz, A., Correia-de-Sá, P., Read, N. D., et al. 2014. Activation of a TRP-like channel and intracellular Ca^{2+} dynamics during phospholipase-C-mediated cell death. J. Cell Sci. 127:3817-3829.
- Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and Western Canada. Phytopathology 86:793-800.
- Guenther, J. F., Michael, K. C., and Nolte, P. 2001. The economic impact of potato late blight on US growers. Potato Res. 44:121-125.
- Haas, B. J., Kamoun, S., Zody, M. C., Jiang, R. H. Y., Handsaker, R. E., Cano, L. M., et al. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. Nature 461:393-398.
- Hansen, Z. R., Everts, K. L., Fry, W. E., Gevens, A. J., Grünwald, N. J., Gugino, B. K., et al. 2016. Genetic variation within clonal lineages of *Phytophthora infestans* revealed through genotyping-by-sequencing, and implications for late blight epidemiology. PLoS One 11:e0165690.
- Haverkort, A. J., Boonekamp, P. M., Hutten, R., Jacobsen, E., Lotz, L. A. P., Kessel, G. J. T., et al. 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Res. 51:47-57.
- Hermansen, A., Hannukkala, A., Naerstad, R. H., and Brurberg, M. B. 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: Mating type, metalaxyl resistance and virulence phenotype. Plant Pathol. 49:11-22.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6:65-70.
- Holt, C., and Yandell, M. 2011. MAKER2: An annotation pipeline and genome-database management tool for second-generation genome projects. BMC Bioinformatics 12:491.
- Hubisz, M. J., Falush, D., Stephens, M., and Pritchard, J. K. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Resour. 9:1322-1332.
- Ingvarsson, P. K., and Street, N. R. 2011. Association genetics of complex traits in plants. New Phytol. 189:909-922.
- Jaime-Garcia, R., Trinidad-Correa, R., Felix-Gastelum, R., Orum, T. V., Wasmann, C. C., and Nelson, M. R. 2000. Temporal and spatial patterns of genetic structure of *Phytophthora infestans* from tomato and potato in the Del Fuerte Valley. Phytopathology 90:1188-1195.
- Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., et al. 2014. InterProScan 5: Genome-scale protein function classification. Bioinformatics 30:1236-1240.
- Judelson, H. S., and Roberts, S. 1999. Multiple loci determining insensitivity to phenylamide fungicides in *Phytophthora infestans*. Phytopathology 89: 754-760.
- Kadamur, G., and Ross, E. M. 2016. Intrinsic pleckstrin homology (PH) domain motion in phospholipase C- β exposes a G $\beta\gamma$ protein binding site. J. Biol. Chem. 291:11394-11406.
- Knapova, G., and Gisi, U. 2002. Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. Plant Pathol. 51:641-653.
- Knaus, B. J., Tabima, J. F., Davis, C. E., Judelson, H. S., and Grünwald, N. J. 2016. Genomic analyses of dominant U.S. clonal lineages of *Phytophthora infestans* reveals a shared common ancestry for clonal lineages US11 and US18 and a lack of recently shared ancestry among all other U.S. lineages. Phytopathology 106:1393-1403.
- Kunze, D., Melzer, I., Bennett, D., Sanglard, D., MacCallum, D., Nörskau, J., et al. 2005. Functional analysis of the phospholipase C gene CaPLC1 and two unusual phospholipase C genes, CaPLC2 and CaPLC3, of *Candida albicans*. Microbiology 151:3381-3394.
- Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Soft. 82. <http://www.jstatsoft.org/v82/i13/>
- Lenth, R. V. 2016. Least-squares means: The R package lsmeans. J. Stat. Softw. 69:1-33.
- Mariette, N., Androdias, A., Mabon, R., Corbière, R., Marquer, B., Montarry, J., et al. 2016. Local adaptation to temperature in populations and clonal lineages of the Irish potato famine pathogen *Phytophthora infestans*. Ecol. Evol. 6:6320-6331.
- Masuta, Y., Kawabe, A., Nozawa, K., Naito, K., Kato, A., and Ito, H. 2018. Characterization of a heat-activated retrotransposon in *Vigna angularis*. Breed. Sci. 68:168-176.
- Matson, M. E. H., Small, I. M., Fry, W. E., and Judelson, H. S. 2015. Metalaxyl resistance in *Phytophthora infestans*: Assessing role of RPA190 gene and diversity within clonal lineages. Phytopathology 105:1594-1600.
- Mizubuti, E. S. G., and Fry, W. E. 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. Phytopathology 88:837-843.
- Ogasawara, H., Obata, H., Hata, Y., Takahashi, S., and Gomi, K. 2009. *Crawler*, a novel Tc1/mariner-type transposable element in *Aspergillus oryzae* transposes under stress conditions. Fungal Genet. Biol. 46:441-449.
- Pecinka, A., Dinh, H. Q., Baubec, T., Rosa, M., Lettner, N., and Scheid, O. M. 2010. Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. Plant Cell 22:3118-3129.
- Petchaboon, K., Srisawad, N., Pongam, P., Chunwongse, C., and Chunwongse, J. 2014. Phenotypic and genotypic structure of *Phytophthora infestans* populations on tomato and potato in the North of Thailand in 2000–2002. Eur. J. Plant Pathol. 140:441-454.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>

- Randall, E., Young, V., Sierotzki, H., Scalliet, G., Birch, P. R. J., Cooke, D. E. L., et al. 2014. Sequence diversity in the large subunit of RNA polymerase I contributes to mefenoxam insensitivity in *Phytophthora infestans*. *Mol. Plant Pathol.* 15:664-676.
- Ribeiro, P. J., Jr., and Diggle, P. J. 2001. geoR: A package for geostatistical analysis. *R News* 1:15-18.
- Saville, A., Graham, K., Grünwald, N. J., Myers, K., Fry, W. E., and Ristaino, J. B. 2015. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. *Plant Dis.* 99:659-666.
- Silverman-Gavrila, L. B., and Lew, R. R. 2001. Regulation of the tip-high $[Ca^{2+}]$ gradient in growing hyphae of the fungus *Neurospora crassa*. *Eur. J. Cell Biol.* 80:379-390.
- Silverman-Gavrila, L. B., and Lew, R. R. 2002. An IP_3 -activated Ca^{2+} channel regulates fungal tip growth. *J. Cell Sci.* 115:5013-5025.
- Silverman-Gavrila, L. B., and Lew, R. R. 2003. Calcium gradient dependence of *Neurospora crassa* hyphal growth. *Microbiology* 149:2475-2485.
- Small, I. M., Joseph, L., and Fry, W. E. 2015. Evaluation of the BlightPro decision support system for management of potato late blight using computer simulation and field validation. *Phytopathology* 105:1545-1554.
- Sujkowski, L. S. 1987. The influence of temperature on *Phytophthora infestans* (Mont.) de Bary. *J. Phytopathol.* 120:271-275.
- Tanghe, A., Carbrey, J. M., Agre, P., Thevelein, J. M., and Van Dijck, P. 2005. Aquaporin expression and freeze tolerance in *Candida albicans*. *Appl. Environ. Microbiol.* 71:6434-6437.
- Tanghe, A., Dijck, P. V., Dumortier, F., Teunissen, A., Hohmann, S., and Thevelein, J. M. 2002. Aquaporin expression correlates with freeze tolerance in baker's yeast, and overexpression improves freeze tolerance in industrial strains. *Appl. Environ. Microbiol.* 68:5981-5989.
- Therrien, C. D., Tooley, P. W., Spielman, L. J., Fry, W. E., Ritch, D. L., and Shelly, S. E. 1993. Nuclear DNA content, allozyme phenotypes and metalaxyl sensitivity of *Phytophthora infestans* from Japan. *Mycol. Res.* 97: 945-950.
- Vose, R. S., Easterling, D. R., Kunkel, K. E., LeGrande, A. N., and Wehner, M. F. 2017. Temperature changes in the United States. Pages 185-206 in: *Climate Science Special Report: Fourth National Climate Assessment*, Vol. I. D. J. Wuebbles, D. W. Fahey, K. A. Hibbard, D. J. Dokken, B. C. Stewart, and T. K. Maycock, eds. U.S. Global Change Research Program, Washington, DC.