Supplementary Materials and Methods

Genomic library preparation and sequencing

Mvc isolate RL1 (*Mvc*-RL1) was previously described (Burchhardt and Cubeta 2014) and isolated as a single conidium from infected shoots of Southern Highbush Blueberry cv. 'Legacy' [Elizabeth × US75 (*V. darrowi*,'Florida 4B', × 'Bluecrop')]) in Ivanhoe, North Carolina.

PacBio RSII sequencing was performed at the University of Florida Interdisciplinary Center for Biotechnology Research (UF-ICBR). Hyphae of *Mvc*-RL1 was grown on a cellophane overlay of 1/2X PDA for 6 days, harvested by scraping the mycelia from the cellophane, and used to isolate high molecular weight (HMW) DNA. DNA quality was evaluated using the Agilent TapeStation with a Genomic Tape. Quantitation was performed by fluorescence (Qubit, ThermoFisher). HMW gDNA was applied to a G-tube (Covaris, Inc.) using fragmentation conditions for 20 kb. Large-insert (20 kb) library construction was performed according to the PacBio protocol (PN 100-938-400-02) with a few modifications. The final UFL library was used for sequencing with the PacBio RS II on two SMRT (Single-Molecule Real-Time) cells.

PacBio Sequel sequencing was performed at the North Carolina State University Genomic Sciences Laboratory (NCSU-GSL). *Mvc*-RL1 mycelial plugs were recovered from storage (-80°C) by plating on Petri dishes containing 1/2X potato dextrose agar (PDA, Difco). Expanding mycelia were subsequently transferred to flasks of 1/2X potato dextrose broth (PDB, Difco). The mycelia in 1/2X PDB were vacuum filtered and stored at -20°C. HMW gDNA was extracted from the mycelia using a CTAB method (Amir *et al.* 2015), and the quantity and quality were determined with Qubit and pulsed-field gel electrophoresis (PFGE), respectively. DNA was separated by size fractionation with the SageELF system (Sage Sciences, Beverly, MA) and a target size of 10 kb. Large-insert (10-20kb) libraries were prepared according to the Pacbio protocol (PN: 100-938-400-03) with size-selection steps omitted, as the input gDNA was previously size-selected using the SageELF system and of an appropriate size for library construction. The sequencing of the final NCSU library was performed with the PacBio Sequel on four SMRT cells.

Genomic Hi-C libraries were prepared by Phase Genomics (Seattle, WA) with the Proximo Hi-C Kit from 0.2g of *Mvc*-RL1 mycelial tissue grown in 1/2X PDB and sequenced at NCSU-GSL on the Illumina NextSeq 500 using 2x150bp PE chemistry.

Genome assembly and gene model prediction

HGap4 (SMRTLink v.4.0) and Canu (v. 1.5) were used to develop draft assemblies with the Sequel and RSII data. Six draft assemblies were generated using RSII only, Sequel only, and combined RSII and Sequel data to develop the draft genome for *Mvc*. The two highest quality genome assemblies were chosen for further comparison, one from Canu and one from HGAP4.

RepeatModeler 1.0.8 (Smit, AFA, Hubley 2015), was used to generate *de novo* repetitive element predictions for the *Mvc* genome using the RMBlast NCBI search engine. The generated repetitive element predictions were combined with the fungal-specific repetitive element libraries in the RepBase database (Bao *et al.* 2015). This combined set of repetitive elements was used by RepeatMasker 4.0.6 (Smit, AFA, Hubley, R & Green 2015) as the search library to mask repetitive elements in the assembled genome and generate a summary table of identified repetitive elements. Gene predictions of two assemblies were conducted using Maker (v. 2.31.8)

(Campbell *et al.* 2014) with Illumina RNA-Seq data described below as the expressed sequence tag (EST) evidence.

BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão *et al.* 2015; Waterhouse *et al.* 2017) was run on predicted gene sets of the two assemblies to assess the completeness of each assembly. Dot plots were generated using MUMmer (v. 3.23) program (Kurtz *et al.* 2004) for comparison of HGAP4 and Canu assemblies. The genome assembly with the largest N50, most discernable nuclear contigs, and highest BUSCO scores was the HGAP4-derived assembly.

Scaffolding by Phase Genomics was performed on only the nuclear contigs of the HGAP4 assembly using Hi-C data. Mis-assembled and mis-oriented contigs in the genome were manually corrected in Juicebox 1.8.8 (Rao *et al.* 2014; Durand *et al.* 2016; Dudchenko *et al.* 2017, 2018).

Functional annotation of gene models

Protein sequences for Maker-predicted genes were imported into Omicsbox (version 1.2) for functional annotation using homology-based searches. The set of gene models were queried against the NCBI NR (Non-Redundant) protein, EggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) Mapper 5.0, and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases. InterProScan was used for annotating sequences based on protein domains and families (Huerta-Cepas *et al.* 2017, 2019).

Parameters used for running gene models against all databases were the default in Omicsbox, except that the E-value cutoff for BLASTp hits was set to 1E-10. BLASTp and eggNOG mapper databases were filtered for fungi. InterProScan and eggNOG annotations were merged with BLAST-based annotation results to build a comprehensive set of functional GO terms for the predicted *Mvc* proteome. Putative effectors for *Mvc* were identified by running EffectorP 2.0 (Sperschneider *et al.* 2016, 2018) on the set of Maker gene models. SMURF (Secondary Metabolite Unique Regions Finder), a web-based software, was used to identify secondary metabolite biosynthesis genes based on genomic clustering and protein domain content (Khaldi *et al.* 2010). Signal peptides were identified in the Maker dataset by using SignalP 5.0 prediction software (Petersen *et al.* 2011; Almagro Armenteros *et al.* 2019b, 2019a). SignalP predicts the presence and location of signal peptide cleavage sites in protein sequences. dbCAN2 was used for predicting CAZymes in *Mvc* (Yin *et al.* 2012; Zhang *et al.* 2018).

Illumina transcriptome sequencing

The Illumina data were developed independently at the UFL Mummy Berry lab (UFL-MBL) and NC State Blueberry Genomics Lab (NCSU-BGL). UFL-MBL data were based on tissues sampled from greenhouse-inoculated blueberry flower styles (3 days post-inoculation (DPI)) and infected stage 1 fruits (20 DPI) of cv. Legacy. The NCSU-BGL data were based on tissue sampled at infected stage 1 and 4 fruits of cv. Arlen, collected at Ideal Tract farm at Castle Hayne, NC. The collected fruits were immediately flash-frozen in liquid nitrogen and kept frozen on dry ice in the field until they were transferred to -80°C freezer.

At UFL-MBL, three biological replications of blueberry styles 3 days post-inoculation, mycelial masses from blueberry fruit 20 days post flower inoculation and hyphae from a sporulating culture grown on oatmeal agar plates (30 g cooked, rolled oat filtrate, 15 g agar per liter) overlaid with a cellophane-membrane were utilized for RNA extraction. Total RNA was extracted using the protocol provided by Norgen Plant/Fungi Total RNA Kit (Norgen Biotek

Corp., Catalog No. 25800) using the DNase I option. The average RNA yield was approximately 1 µg/µl in a 30 µl volume. Total RNA samples were processed at the UF-ICBR for quality analysis, library construction, and transcript cDNA sequencing. RNA concentration was determined on Qubit® 2.0 Fluorometer (ThermoFisher/Invitrogen, Grand Island, NY), RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Total RNA with 28S/18S > 1 and RNA integrity number (RIN) ≥ 7 were used for RNA-seq library construction. mRNA was isolated using NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs, Catalog No. E7490) and RNA library construction with NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Catalog No. E7530) according to the manufacturer's user guide. Barcoded libraries were sized on a Bioanalyzer (Agilent Technologies, Santa Clara, CA), quantitated with the QUBIT fluorometer. A 200-1000 broad library peak was observed for each sample. Quantitative PCR was used to validate library functionality, using the KAPA library quantification kit (Kapa Biosystems, Catalog No. K4824). The nine individual samples were pooled equimolarly except samples 1-3 (3 DPI flower inoculations) were doubled to partially offset the lower ratio of fungal to plant tissue in these replicated samples. Sequencing was performed on two lanes of the Illumina HiSeq3000 instrument using the clustering and sequencing reagents provided by Illumina (San Diego, CA, USA). Paired-end, 2x100 bp cycle runs required combined adding of reagents from the 150 cycles and the 50 cycles kits (Catalog No. FC-410-1002, FC-410-1001, and PE-410-1001).

At NCSU-BGL, total RNA was extracted from flash-frozen tissues using the Sigma-Aldrich Spectrum™ Plant Total RNA Kit according to manufacturer's instructions. The transcriptome libraries for infected Arlen tissues were developed at NCSU-BGL using Bioo Scientific (Austin, TX) NEXTflex® Rapid Illumina Directional RNA-Seq Library Prep Kit (Catalog No. NOVA-5138) according to manufacturer's instructions. To multiplex, these libraries were barcoded using the Illumina-compatible Bioo Scientific (Austin, TX) NEXTflex® DNA Barcodes - 6 kit (Catalog No. NOVA-514101). Sequencing was done on the Illumina HiSeq 4000 at Novogene (Sacramento, CA). CLC Genomics Workbench (v. 10.1.1) was used to trim datasets for adapters, and low-quality reads.

Comparative analyses

The *Mvc* genome was compared to genomes of close relatives in the taxonomic family Sclerotiniaceae, including *Botrytis cinerea*, *Monilinia fructicola*, *Monilinia fructigena*, *Monilinia laxa*, and *Sclerotinia sclerotiorum*, using the python version of MCScan (Wang *et al.* 2012). Microsynteny comparisons of the *Mvc* and *S. sclerotiorum* mating-type (MAT) loci were conducted using the Artemis Comparison Tool (ACT) (Carver *et al.* 2005). Analyses for protein homology of MAT locus genes were conducted using BLASTp.

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