

Phytophthora ramorum related submissions

2019 American Phytopathological Society meeting, Cleveland, August 3 – 7, 2019

ABSTRACTS

Epidemic dynamics of multiple introductions of the sudden oak death pathogen into Oregon forests

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Sudden oak death (SOD) has been actively managed in Oregon since the early 2000s. There are currently four recognized clonal lineages, with the NA1 lineage being primarily responsible for the epidemic in southwest Oregon forests. We compared population dynamics of the NA1 *Phytophthora ramorum* outbreak first reported in 2001 to an outbreak of an emerging EU1 *P. ramorum* clonal lineage first detected in 2015. We also tested if NA1 was reintroduced since the initial documentation of two introductions. Infested regions of the forest were sampled between 2013-2018 (n=903) and screened at 15 microsatellite loci. Most genotypes were transient, with 272 of 358 unique genotypes emerging one year and disappearing the next. Diversity of EU1 was very low and isolates were geospatially clustered (<8 km apart), suggesting a single EU1 introduction. Some forest isolates are genetically similar to isolates collected from a local nursery in 2012, suggesting introduction of EU1 from this nursery or simultaneous introduction to both the nursery and latently into the forest. In contrast, the older NA1 populations were more polymorphic and spread over 30 km. Principal component analysis supported two to four independent NA1 introductions. The NA1 and EU1 epidemics infest the same area but show disparate demographics owing to initial introductions spaced 10 years apart. Comparing these epidemics provides novel insights into patterns of emergence of clonal pathogens in forest ecosystems.

Dual Transcriptome Analysis Reveals Insights into Innate and Phosphite-Induced Resistance of Tanoak to *Phytophthora ramorum*

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Phosphites have been used in the control of sudden oak death, however, the precise mode of action of these compounds is not fully understood. We designed an inoculation experiment on four open-pollinated tanoak families, previously defined as partially resistant. Stems of treatment-individuals were sprayed with phosphite, and 7 days later distal leaves were inoculated with the sudden oak death pathogen *Phytophthora ramorum*. Leaves from treated and untreated control plants were harvested for RNA extraction before and seven days after inoculation, and transcriptomes of both host and pathogen were analyzed. We found that sets of genes associated with innate resistance and with phosphite-induced resistance largely overlapped within a more susceptible but phosphite-treatment responsive tanoak family, supporting the hypothesis that phosphite treatment increases the resistance of susceptible host plants to *Phytophthora* infection. In addition, we found that genes of the pathogen involved in detoxification, such as ATP-binding cassette (ABC) transporters and vitamin B6 biosynthesis genes, were upregulated in phosphite-treated plants, but not in untreated plants. In summary, our dual RNA-Seq supports a dual mode of action of phosphite compounds, including a direct toxic effect on *P. ramorum* and an indirect enhancement of resistance in the tanoak host.

Copy number variation occurs in all four clonal lineages of the sudden oak death pathogen, *Phytophthora ramorum*

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Phytophthora ramorum causes sudden oak death throughout the American west coast, where it infects tanoak, coast live oak, and California bay laurel, as well as in the UK, where it infects Japanese larch. Copy number variation (CNV) has been implicated as contributing to pathogenic phenotypes in clonal pathogens including *P. ramorum*, but it has not been determined how extensive CNV may be in *P. ramorum* populations. In order to explore this topic we first updated the genomic reference by sequencing Pr-102 using Pacific Biosciences technology. We then downloaded 189 read libraries from the SRA and sequenced 11 genomes from southern Oregon to explore copy number variation within three of the clonal lineages (NA1, NA2, EU1) as well as variation in southern Oregon. The updated reference resulted in a 75,183,546 bp assembly with no gaps, over 20 Mbp longer than the reference produced by the JGI using Sanger sequencing which was shorter and contained gaps. The assembly had a N50 of 1,287,631 indicating the fewer contigs were longer than the JGI reference that had a N50 of 308,042. While most isolates were observed to be predominantly diploid, copy numbers of three, four, and possibly five were observed for large contiguous regions in many but not all strains. CNV may thus be the predominant form of genetic diversity generation in clonal lineages. Our results suggest that CNV occurs in almost all lineages of *P. ramorum* and may play an important role in *P. ramorum* epidemics worldwide.

Is an invasive forest pathogen moving into a new environment? *Phytophthora ramorum* found on chaparral plants in Coastal California

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Phytophthora ramorum, causal pathogen of Sudden Oak Death and ramorum blight, was discovered in California in the mid-1990s, and spread quickly along a narrow band close to the coast characterized by mild temperatures and abundant year-long moisture [Ed. Note - fog in summer]. The foliar host California bay laurel (*Umbellularia californica*) is an essential driver for the spread of the disease to 'dead-end hosts', such as coast live oak (*Quercus agrifolia*). Recently, *P. ramorum* was detected on several chaparral plants (manzanita, *Arctostaphylos* spp.; chaparral pea, *Pickeringia montana*) on a high, sun-exposed ridge in Marin County. During 2018, a severe outbreak of disease was observed on Mt. Tamalpais, with symptoms including wilting, branch dieback and occasionally plant death. Several plants showed a positive reaction for *Phytophthora* spp. using immuno-strips; and *P. ramorum* was detected using PCR from a manzanita stem. In addition, *Neofusicoccum australe* (Botryosphaeriaceae) was isolated from a symptomatic plant. Potted rhododendron plants were placed near symptomatic plants on Mt. Tamalpais to monitor the possible spread of airborne inoculum and the effect of environmental parameters such as rainfall on the timing and appearance of disease symptoms. While it is still unclear whether the observed symptoms are caused by a disease complex, and which role *P. ramorum* has in it, mounting evidence indicates that *P. ramorum* is expanding its host range and moving into new environments.

High-throughput sequencing of *Phytophthora ramorum* Ex-type using long-read MinION nanopore sequencing

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Sequence information of type specimens is critical in order to make correct molecular identification and develop robust diagnostic systems. *Phytophthora ramorum* is one of the species of biosecurity concern in the United States, United Kingdom and other areas of the world. Due to the significance of the species there are currently 22 genome sequences for isolates of *P. ramorum* deposited at the NCBI, however a whole genome sequence for the Ex-type of *P. ramorum* is not available. Here we report the whole-genome sequence for the Ex-type of *P. ramorum* generated using High Throughput Sequencing on the MinION platform from Oxford Nanopore Technology. The quality filtered 625,764 reads were assembled in to 1322 contigs indicated extended genome size of 52.4 Mb. We analyzed our Nanopore assembly of the *P. ramorum* (CPHST-BL 55G which is a duplicate of specimen P10103) with another isolate CC1011 of EU1 lineage available at the NCBI, and found 251,859 quality Single Nucleotide Polymorphisms (SNPs) genome-wide. When compared to the genome of isolate SOD69/12 of EU2 lineage 441,859 SNPs were found. Completion of Ex-type draft genome of *P. ramorum* is highly important since this is the representative specimen for the species of biosecurity concern at different regions of the world.