# Next Generation Sequencing of Oomycete Communities in Nursery Irrigation Water[1]

**Joyce Eberhart,[2] Fumiaki Funahashi,[2] Zachary S. L. Foster,[2] and Jennifer Parke[2]**

## Abstract

Horticultural nurseries are under increasing pressure to reduce, remediate, and recycle irrigation water. A major constraint for reusing irrigation water is contamination by waterborne plant pathogenic *Phytophthora* and *Pythium* species. Current research is focused on helping plant nurseries monitor oomycete pathogens in their irrigation water to determine the need for water treatment, evaluate effectiveness of treatment options, and enable selection of cost-effective ways to disinfest water.

A sensitive method to identify waterborne oomycetes present at low concentrations was needed. A semi-quantitative method that would allow for determining the relative frequency of diverse, co-occurring species was also desired (Parke and others 2014). Next generation sequencing has the potential to accomplish both goals. Vannini and others (2013) developed an ITS6 and ITS7 primer set that was successful at amplifying oomycetes with the Roche 454 platform. Because Illumina has higher throughput capabilities and is becoming the standard for next generation sequencing, we investigated modifications that would allow us to use the same primers with the Illumina MiSeq platform. Each primer was designed with the appropriate Nextera XT adapter and the forward primer included a spacer designed to raise the annealing temperature to the recommended level. Primer sequences were as follows:

MiSeq ITS6: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG K
GAAGGTGAAGTCGTAACAAGG 3'

MiSeq ITS7: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
AGCGTTCTTCATCGATGTGC 3'

For detection of waterborne oomycetes, water samples (1 L) were collected from various locations in a nursery and filtered through 5µm Millipore filters. DNA was extracted directly from filters using beadbeating followed by a chloroform/phenol extraction. PCR was performed using Platinum™ taq and PCR product was submitted to the Oregon State University Center for Genome Research and Biocomputing (CGRB) Core lab. The CGRB Core lab performed cleanup of the PCR product and attached the barcodes using the Nextera XT Index kit. Samples were normalized and pooled before quality controls were performed with qPCR and a Bioanalyzer. Sequencing was performed on the Illumina MiSeq on a 250 bp paired-end run.

Sequence data processing was done using QIIME v. 1.8.0. Only forward reads were used for a preliminary analysis. Sequence bases with quality scores >25 were retained and chimera detection was conducted with the "usearch61" method after primer sequences were removed. Curated sequences were grouped into operational taxonomic units (OTUs) at a similarity level of 99% by uclust algorithms.

[1] A version of this poster was presented at the Sixth Sudden Oak Death Science Symposium, June 20-23, 2016, San Francisco, California.

[2] Oregon State University, Corvallis, OR 97331.
Corresponding author: Joyce.Eberhart@oregonstate.edu.

Oomycete reads were queried by BLAST algorithms against a reference dataset with *Phytophthora* sequences downloaded from Phytophthora-ID.org and other oomycete sequences used in Robideau and others (2011). The R programming environment (www.R-project.org) was used for downstream analysis.

To ensure that we can detect multiple species in water and accurately determine their relative abundance, we created mock communities composed of 11 *Phytophthora* and *Pythium* species. DNA extracts of cultures of each species were quantified and combined to determine if their relative abundance in the mixture would be reflected in the sequence analysis. Extracts diluted to 5 ng/µl DNA were combined in equal amounts and pooled. PCR performed with the pooled samples showed that many species had similar numbers of reads, but PCR bias may be observed for some species (fig. 1). We then pooled these extracts using 20x greater amounts of DNA from *Phytophthora syringae* and found that the number of reads for this species was approximately 16x the average number of reads for the other species (fig. 2). We also separately amplified each species and the resulting amplicons were combined at 5 ng/µl each. The relative abundance of the number of reads was mostly similar for these samples combined equally post-PCR (fig. 3).

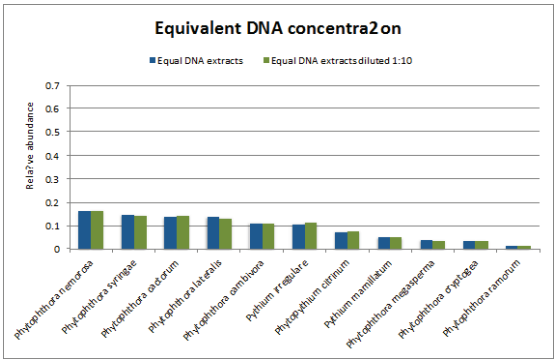


Figure 1—DNA extracts (5 ng/µl) were combined in equal amounts and pooled. PCR was performed with the pooled samples and with a 1:10 dilution of the pooled samples.

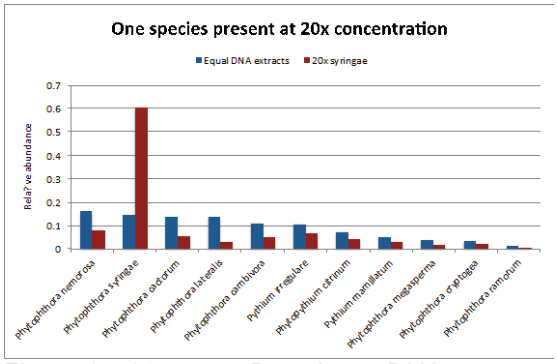


Figure 2—20x more *P. syringae* DNA was combined with the DNA of other species. The number of reads of *P. syringae* was approximately 16x the average number of reads for the other species.

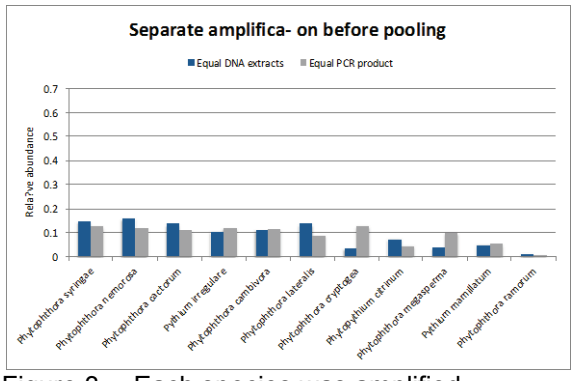


Figure 3— Each species was amplified separately and the resulting amplicons were combined at 5 ng/µl each.

For a subset of the water samples, we compared the detection sensitivity for DNA extracted directly from filters with extraction from rhododendron leaf baits. Leaf baits were incubated in water samples for 3 days followed by incubation in moist chambers for an additional week. Up to five 6-mm disks were taken

from lesioned areas and stored in silica gel at room temperature (Ockels and others 2007). DNA was extracted from the leaf disks with the Synergy™ extraction kit. Amplification procedures were the same as for extractions from filters. Samples obtained using different detection methods (filtered water or baited leaves) and the mock community were used to generate a non-metric multidimensional scaling plot of oomycete OTU communities. The results show distinct community assemblages of oomycetes among the two different detection methods and the mock community (fig. 4).

Once protocols have been fully developed and validated, Illumina sequencing has the potential to be a sensitive method to detect, identify, and estimate the relative abundance of oomycete communities from water samples. This knowledge will be used to help nursery managers make informed decisions about effective water disinfestation strategies, reducing the risk of establishment of plant pathogens.

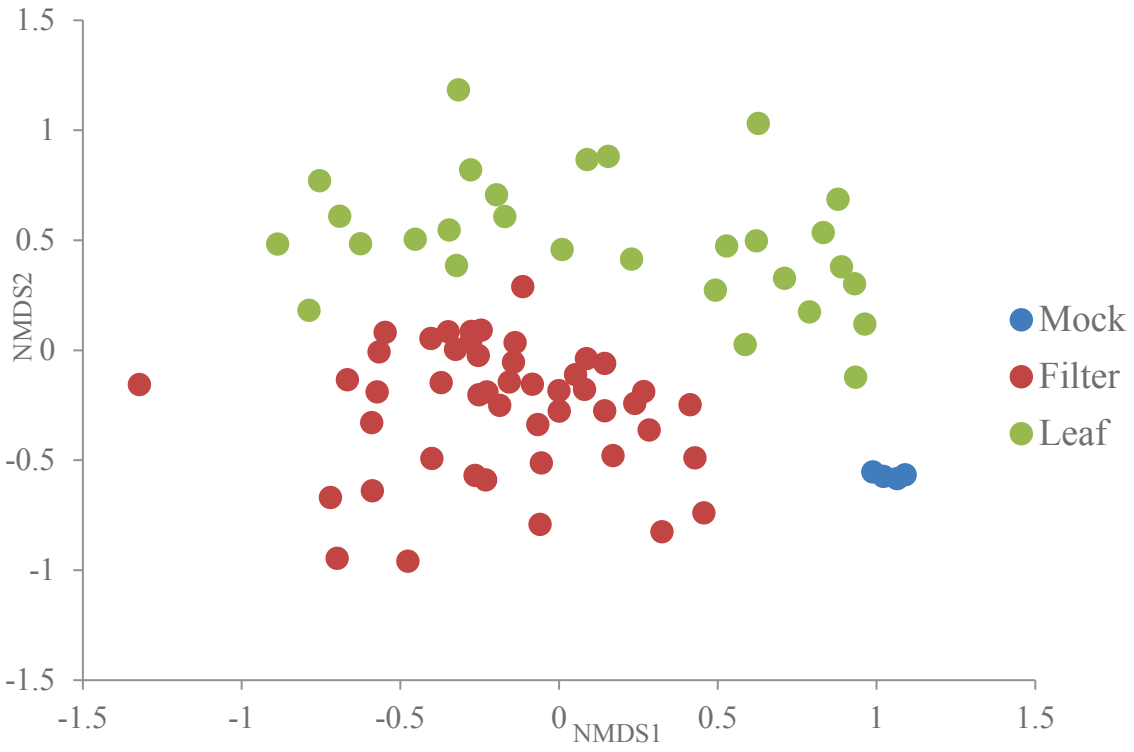


Figure 4—OTU abundance-based ordination of samples (each dot represents the oomycete community sequenced from an individual sample).

## Literature Cited

**Ockels, F.S.; DiLeo, M.V.; Bonello, P. 2007**. Desiccation at ambient temperature effectively preserves plant tissues infected with Phytophthoras. Online. Plant Health Progress. doi:10.1094/PHP-2007-0302-01-RS.

**Parke, J.L.; Knaus, B.J.; Fieland, V.J.; Lewis, C.; Grünwald, N.J. 2014.** *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. Phytopathology. 104: 1052–1062.

**Robideau, G.P.; De Cock, A.W.A.M.; Coffey, M.D.; Voglmayr, H.; Brouwer, H.; Bala, K.; Chitty, D.W.; Desaulniers, N.; Eggertson, Q.A.; Gachon, C.M.M.; Hu, C.H.; Kupper, F.C.; Rintoul, T.L.; Sarhan, E.; Verstappen, E.C.P.; Zhang, Y.; Bonants, P.J.M.; Ristaino, J.B.; Levesque, C.A. 2011.** DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molecular Ecology Resources. 11: 1002–1011.

**Vannini, A.; Bruni, N.; Tomassini, A.; Franceschini, S.; Vettraino, A.M. 2013.** Pyrosequencing of environmental soil samples reveals biodiversity of the *Phytophthora* resident community in chestnut forests. FEMS Microbiology Ecology. 85:433–442.