Phytophthora Sampling Report

2023

STOCKTON EAST WATER DISTRICT

REFORMATTED BY THE STOCKTON EAST WATER DISTRICT, BASED ON THE PHYTOPHTHORA SAMPLING REPORT OF GREG BROWNE[1], NATALIA OTT[1], AND MOHAMED NOURI[2] ([1]USDA-ARS, DAVIS AND [2]UC COOPERATIVE EXTENSION, STOCKTON)

Introduction

In order to comply with the Sustainable Groundwater Management Act, the Stockton East Water District (SEWD) is encouraging walnut and cherry growers to use surface water for irrigation. However, many growers are still hesitant to irrigate with surface water because of concerns that it might introduce Phytophthora into their orchards. In the late 1980's, surveys for Phytophthora detected plant pathogenic species in the waterways supplying the SEWD (Mircetich et al., 1985), but no recent surveys for the pathogen have been conducted in the district's water.

This project gathered data to help assess concerns over the potential presence of pathogenic Phytophthora species in SEWD surface water. Specifically, we 1) surveyed SEWD waterways throughout the irrigation season for the presence of Phytophthora species, 2) compared Phytophthora populations in soil from orchards irrigated with SEWD surface water versus orchards irrigated with groundwater, and 3) determined whether on-farm irrigation systems can bring viable Phytophthora inoculum from the waterway into the orchard.

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Significant FINDINGS

Objective 1. Survey SEWD waterways throughout the irrigation season for the presence of Phytophthora species

- Phytophthora species were found throughout sampled SEWD waterways
- Many of the species detected are known pathogens of walnut, cherry, and other local crops
- Known pathogens of walnut and cherry were found throughout the irrigation season

Objective 2. Compare Phytophthora populations in soil from orchards irrigated with SEWD surface water versus orchards irrigated with groundwater

- Phytophthora species were detected in 25-50% of sampled orchards
- Surface water irrigated orchards and groundwater irrigated orchards had the same proportion of positive Phytophthora detections

Objective 3. Determine whether on-farm irrigation systems can deliver viable inoculum of Phytophthora from a surface waterway into an orchard

- Viable Phytophthora was detected coming into orchards from SEWD waterways through both drip emitters and solid set sprinklers

*The Principal Investigator, G.T. Browne, would like to acknowledge that N. J. Ott completed this work, its data analyses, and its report draft as part of her Ph.D. thesis.

Methods

OBJECTIVE 1

Objective 1. Survey SEWD waterways throughout the irrigation season for the presence of Phytophthora species

Water was collected during the irrigation season from waterways making up the Stockton East Water District (SEWD) (Figure 1, Table 1). Six sites distributed along the Calaveras River, Mormon Slough, Mosher Creek, and Potter Creek, referred to as "core sites", were sampled five times: in June, early-July, mid-July, September, and October 2022. At the mid-July and October sampling dates, an additional nine sites, referred to as "expanded sites", were sampled along with the core sites on the same waterways, making for a total of 15 sampling locations on these dates. At each sampling time, two water subsamples were collected from each site, with the exception that Site #3 had no water to collect for the October sampling (Table 1). In total, 94 subsamples were collected over the course of the study. Subsamples were collected from the waterway to a depth of no more than 15 cm using a 19-L bucket lined with a plastic bag. After collection, subsamples were transported back to the laboratory and 2 to 9 L of water from each subsample were filtered through a 100-µm nylon mesh filter, a 20-µm nylon mesh filter, and two 5-µm nitrocellulose filters. After filtration, the two 5-µm nitrocellulose filters for each subsample were stored at -20 °C until further processing. During the filtering of the mid-July samples a negative control was produced by filtering 9 L of autoclaved DI water as described for the SEWD samples.

After all sample collection was complete, the nitrocellulose filters for each subsample and the control were ground into a fine powder, and total community DNA was extracted from 100 mg of the ground filter from each using the Dneasy Plant Pro kit (Qiagen, 69204). A protocol using the published primers Oom18s (Legeay et al., 2019), 18PPh2F and 28Ph2R (Scibetta et al., 2012) was developed for amplifying the full ITS region (ITS1-5.8s-ITS2) of the ribosomal RNA gene of Phytophthora from total community DNA. PCR was performed using a semi-nested approach; a first PCR using the forward primer Oom18s and the reverse primer 28Ph2R was followed by a second PCR using primers 18Ph2F and 28Ph2R with 8-bp barcodes and 2-bp linkers added to each primer. Each subsample and control were given a unique combination of forward and reverse barcodes to allow for tracking subsample identity throughout analysis.

After PCR, 10 µL of each subsample was examined on an agarose gel, the brightness of the target band for each subsample was visually compared, and subsamples were pooled into three categories: bright target amplification (three subsamples), faint target amplification (45 subsamples), and no visible target amplification (46 subsamples). Each of these three pools was purified and concentrated using the Wizard SV Gel and PCR clean-up kit (Promega), then the entire volume of each pool was run on a 1% agarose gel and the target band (or expected target band location) was excised and extracted using the same kit. Finally, DNA concentration was determined for each of the three pools using a Qubit dsDNA HS assay (Thermo Fisher Scientific, USA). For the sequencing submission, the entire volumes of the "faint amplification" and "no amplification" pools were mixed, and the average amount of DNA per sample was calculated. An equivalent amount of DNA per sample was added from the "bright amplification" pool. This final library of all subsamples was submitted to the UC Davis DNA Technologies Core for library preparation and sequencing using the PacBio SMRT-cell Sequel II sequencing technology.

Sequences were demultiplexed using the program “demultiplex” (Laros) to yield a single fastq file per sample. Further sequence processing was done using the R statistical software (R Core Team, 2021) in the RStudio environment (RStudio Team, 2022) using the dada2 package (Callahan et al., 2016) and following the published tutorial for processing PacBio sequences (Callahan et al., 2019). Species assignments were done manually using NCBI BLAST, with results limited to voucher sequences or sequences deposited by IDphy (Abad et al., 2022).

Data analysis was conducted using a combination of the R statistical package and PRIMER version 7 (Clarke & Gorley, 2015). Sequence data were imported into R using phyloseq (Mcmurdie & Holmes, 2013), any non-Phytophthora amplicon sequence variants (ASVs) were removed, and remaining ASVs from Phytophthora were collapsed to the level of species. These data were then used in two different ways: for rarefaction and detection tables in R, and, after additional preprocessing in R, for statistical analysis of variance in PRIMER.

For detection tables, the species-level data were split into two sets: the core set, which included subsamples from each of the core sites taken at each of the five sampling times, and the expanded set, which included subsamples from the core sites and the additional sites at the mid-July and October sampling times. The mirlyn package (Cameron et al., 2021) was used to determine the appropriate rarefaction level for each set of subsamples: 237 reads for the core set and 139 reads for the expanded set. Each set was rarefied to the appropriate number of reads, and then the two subsamples for each site at each sampling time were averaged before generating detection tables.

For statistical analysis, subsamples in the species-level data with fewer than 128 reads were removed, and the remaining subsamples were normalized using cumulative sum scaling implemented in metagenomeSeq (Paulson et al., 2013). In these normalized data, the two subsamples for each site at each sampling time were averaged, and the data were imported into PRIMER, where a 4th root transformation was applied to the normalized reads in each averaged sample. Because of the number of samples that had been removed by this stage in the analysis due to zero or near-zero numbers of reads, it was necessary to select balanced sets of samples for the following analyses: 1) a core set analysis that included taxa from Site #1 through Site #4 from the first four sampling times (June, early-July, Mid-July, and September); 2) an expanded set analysis that included taxa from Site #2, Site #7, Site #10, Site #13, and Site #15 sampled in mid-July and October.

The two balanced datasets were analyzed for community-level differences between sites and between sampling times. Bray-Curtis dissimilarity matrices (based on counts of each distinct Phytophthora species in a sample) were produced for each set, and the dissimilarities were subjected to permutational analysis of variance (PERMANOVA) with 9,999 permutations. The PERMANOVA model specified Site and Sampling Date as fixed factors. Significant results in the global PERMANOVA were followed up by pairwise tests, as appropriate. The Shannon Diversity Index (a measure of the diversity of species in a population or sample) was calculated for each sample. The Shannon Diversity Indices were then used to generate a Euclidean-distance-based dissimilarity matrix for samples within the core set and, separately, for samples within the expanded set. The core and expanded set dissimilarity matrices were, in turn, subjected to PERMANOVA (9,999 permutations, Site and Sampling Date as fixed factors) to test for differences in alpha diversity between sites and between sampling times.

Methods

OBJECTIVE 2

Objective 2. Compare Phytophthora populations in soil from orchards irrigated with SEWD surface water versus orchards irrigated with groundwater

Soil sampling was conducted in 40 orchards within the SEWD. Orchards were predominantly walnut and cherry, and in a few cases almond or apple orchards were sampled as well. Irrigation water source (surface waterway, groundwater, or a mix of the two) as reported by the grower or manager was recorded for each orchard.

Two soil subsamples were collected from each orchard, for a total of 80 soil subsamples. Each of the subsamples was comprised of soil collected to a depth of approximately 30 cm using a hand auger at five locations in the orchard; the soil was pooled and mixed, and a total of approximately 2 L of soil was saved per subsample. After collection, soil subsamples were transported back to the lab, where they were left at 20 to 25° C in open bags and mixed every few days until they were dry enough to homogenize by hand. A 50-mL aliquot was collected from each dried, homogenized subsample and stored at -80° C for processing and PacBio sequencing. The remainder of each subsample was then sealed in its bag and stored at 20 to 25° C until all samples were collected, dried, homogenized, and ready for baiting.

Two days prior to baiting, each soil subsample was moistened with autoclaved DI water and incubated at room temperature. On the day of baiting, each subsample was flooded with tap water and baited with two green Bartlett pears and three rhododendron leaves. The flooded subsamples and baits were incubated at room temperature for 48 hours, then the baits were removed and rinsed with tap water. Rhododendron leaf baits were cut into 0.5-cm squares and four squares from each leaf (12 squares total per subsample) were embedded in petri plates containing corn meal agar amended with pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene (PARP) (Jeffers, 1986). The remaining leaf squares were frozen and stored at -80 °C for processing and PacBio sequencing. Pear baits were incubated at room temperature for 7 days and monitored daily for the appearance of lesions. A small piece of each lesion was excised and embedded in PARP medium.

The PARP plates from both leaf and pear cultures were monitored over the next 7 days for growth of Phytophthora. Isolates from each soil subsample were grouped based on colony morphology and microscopic structures produced in culture, and representatives from each group were selected for DNA sequencing. A diagnostic region of DNA (ITS of the rRNA gene) was amplified from each representative using the PCR primers ITS6 (Sapkota & Nicolaisen, 2015) and ITS4 (White et al., 1990). PCR product was cleaned using ExoSAP (Exonuclease 1 and Shrimp Alkaline Phosphatase) (New England Biolabs, Ipswich, MA), then submitted for Sanger sequencing at the UCDNA Sequencing Facility. Upon receipt, species assignments were done manually for each sequence using NCBI BLAST, with results limited to voucher sequences or sequences deposited by IDphy.

The frozen soil aliquot and frozen leaf squares from each soil subsample were ground to a fine powder, and total DNA was extracted from each using the Dneasy Powersoil kit (soil) and Dneasy Plant Pro kit (leaf squares). As in Objective 1, PCR was performed with the primers Oom18s, 18PPh2F, and 28Ph2R; subsamples were pooled according to band brightness on an agarose gel, and for each pool the expected target band was excised and extracted. Finally, the pools were quantified, combined, and submitted to the UC Davis DNA Technologies Core for library preparation and sequencing using the PacBio SMRT-cell Sequel II sequencing technology. PacBio sequence processing was performed as in Objective 1, using a combination of the "demultiplex" program and dada2 in R, with species assignments done manually using NCBI BLAST.

Orchard soil was considered positive for Phytophthora if 1) at least one Phytophthora isolate was obtained by baiting using either bait material from either soil subsample from the orchard, or if 2) the number of sequencing reads for any one species of Phytophthora exceeded a predetermined threshold in any of the samples associated with that orchard, i.e. in the frozen leaf bait or the frozen soil aliquot of either subsample from an orchard. The data were analyzed using a threshold of 500 reads (least stringent), 1,000 reads (moderately stringent), and 10,000 reads (most stringent). Orchards were classified as being irrigated with either groundwater or surface water. Orchards irrigated with a mix of groundwater and surface water were classified as surface water orchards. A Chi Squared test was used to determine if the proportion of Phytophthora-positive orchards differed between the two sources of irrigation water.

Methods

OBJECTIVE 3

Objective 3. Determine whether on-farm irrigation systems can deliver viable inoculum of Phytophthora from a surface waterway into an orchard

Baiting was used to detect viable Phytophthora in irrigation water as it was applied to the orchard via irrigation emitters. This was done in five orchards which are summarized in Table 2. These orchards represented both surface water and groundwater irrigation applied using either full-coverage sprinklers or drip emitters. During a normal irrigation, baiting was conducted by placing three green Bartlett pears in a 40-cm x 60-cm plastic tote with a screen lid. Sampling totes were placed on a tarp over an upturned plastic bin to prevent soil and water from splashing from the orchard floor into the tote. Five sampling totes were set out in each orchard prior to the start of irrigation and positioned to collect water from a single sprinkler or drip emitter. Pears and totes remained in the orchard throughout the irrigation and were collected within 2 to 4 hours after the water was shut off. The amount of water collected in each tote was recorded. For orchards irrigated with surface water, baiting was also conducted in the waterway near the irrigation system intake point. This was done by placing Bartlett pears and pieces of sytrofoam into small mesh bags, which were floated in the waterway near the irrigation intake point for the duration of the irrigation.

After collection, pears were transported back to the laboratory and incubated at room temperature for 7 days and monitored daily for the appearance of lesions. A small piece of each lesion was excised and embedded in PARP medium, where it was monitored for growth of Phytophthora. Isolates were grouped based on colony morphology and microscopic structures produced in culture, and representatives from each group were selected for DNA sequencing. Diagnostic ITS DNA was amplified from the rRNA gene of each representative using the PCR primers ITS6 and ITS4. PCR product was cleaned using ExoSAP, then submitted for Sanger sequencing at the UCDNA Sequencing Facility. Upon receipt, species assignments were done manually for each sequence using NCBI BLAST, with results limited to voucher sequences or sequences deposited by IDphy.

Results & DISCUSSION

OBJECTIVE 1

Objective 1. Survey SEWD waterways throughout the irrigation season for the presence of Phytophthora species

During the sampling period, over 30 species of Phytophthora were detected in the SEWD waterways (Tables 3 and 4). These included several species known to be aggressive pathogens of walnut, cherry, or other crops grown in the area, as well as many riparian Phytophthora species, which are either not reported as pathogens or are only known as weak pathogens of non-crop hosts. Among the species of Phytophthora that were detected in SEWD waterways, the important pathogens on walnut or cherry were: Phytophthora cactorum, Phytophthora cambivora, Phytophthora citrophthora, Phytophthora pini, and a species which could not be identified clearly based on the ITS rDNA, but was equally similar to Phytophthora citricola, Phytophthora pini, and Phytophthora plurivora. Phytophthora cactorum is widely distributed in world horticultural settings and causes crown rot and scion cankers on many deciduous fruit and nut trees, including walnut, cherry, and other species of Prunus. Phytophthora citrophthora, though not as widely distributed in deciduous orchards as P. cactorum, has been associated with crown rot and scion cankers on both cherry and walnut in California. Phytophthora pini and several closely related species formerly known as P. citricola can be very aggressive root crown and scion pathogens of walnut and Prunus species.

Among the core sites, samples from the Calaveras River (site 1, 2 and 4) and from Mosher Creek (site 3) had the largest number of Phytophthora species known as pathogens on walnut and cherry (Table 3). However, every core site hosted at least one walnut or cherry pathogen over the course of the summer. PERMANOVA analysis of the Phytophthora assemblages at the core sites indicated that these assemblages varied significantly by site as well as sampling time (Site: $P < 0.001$ and Pseudo-F = 2.8; Sampling time: $P = 0.02$, Pseudo-F = 2.0). In spite of the changes over time, Phytophthora species pathogenic on walnut or cherry were found in the waterways from June through September.

Results & DISCUSSION

OBJECTIVE 1 - CONTINUED

The October samples had to be eliminated from this analysis due to low total number of Phytophthora detections, and no crop-pathogenic Phytophthora species were detected in the core sites during this sampling time.

Among the expanded sites, samples from the Calaveras River, Mosher Creek, and Potter Creek had the largest number of Phytophthora species known as pathogens on walnut and cherry (Table 4). Most of these detections were from the mid-July sampling time. At the October sampling time there were no detections for Phytophthora species known to be highly aggressive on walnut or cherry, and few crop-pathogenic Phytophthora species were detected. PERMANOVA analysis indicated that the Phytophthora assemblages at the expanded sites varied significantly by sampling time ($P = 0.04$, Pseudo-$F = 3.3$) but not by site ($P = 0.37$ and Pseudo-$F = 1.1$).

Taken together, these results indicate that Phytophthora species are commonly found in the SEWD waterways, including several species that are pathogenic on walnut, cherry, and other crops irrigated with SEWD surface water. This is consistent with the results of a baiting study conducted in the area in the late 1980s (Mircetich et al., 1985). The assemblage of Phytophthora detected at sampling sites varied throughout the summer, but Phytophthora species considered to be aggressive on walnut and cherry were found from early June through mid-September, and it was not until the very end of the irrigation season in mid-October that we saw a substantial decrease in the number of aggressive species detected. It is important to be aware, however, that this study provided primarily a qualitative and not a quantitative view of the Phytophthora species found among the locations and times surveyed. It is also important to remember that these samples are from a single season of sampling and may not represent the Phytophthora populations found in SEWD waterways from year to year. Phytophthora assemblages are highly variable in both space and time, so any comparisons of Phytophthora populations between waterways would require more sampling to confirm.

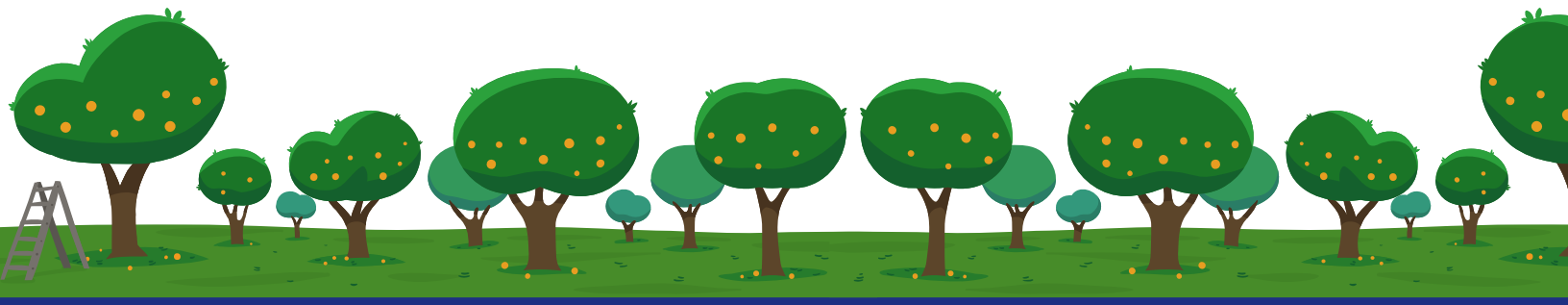
Results & DISCUSSION

OBJECTIVE 2

Objective 2. Compare Phytophthora populations in soil from orchards irrigated with SEWD surface water versus orchards irrigated with groundwater

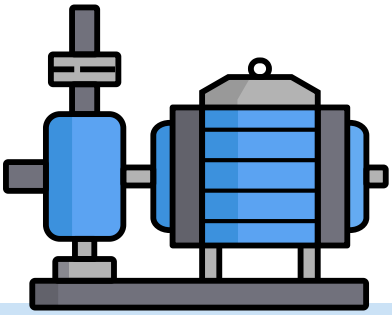
No significant differences were found between the proportion of positive Phytophthora detections in groundwater-irrigated or surface water-irrigated orchards. Pear and leaf baiting of orchard soils, followed by culturing and ITS-based sequencing to identify species of Phytophthora, indicated four groundwater orchards and three surface water orchards as positive for Phytophthora. The culture-independent approach, which used PacBio sequencing directly from the soil or from frozen leaf bait tissue, revealed an additional 1 to 5 groundwater orchards and an additional 2 to 4 surface water orchards positive for Phytophthora, depending on the stringency of the cut-off used to determine a positive detection. Combining both the baiting results and the culture-independent results, at the lowest-stringency cut-off 9 out of 20 groundwater and 8 out of 20 surface water orchards were positive for Phytophthora (42.5% positive). At the highest-stringency cut-off, 5 out of 20 groundwater and 5 out of 20 surface water orchards were positive for Phytophthora (25% positive). The combined results for Phytophthora detections by both culture-based and culture-independent methods are summarized in Table 5. Using this combination of methods, in surface water orchards, Phytophthora cinnamomi, Phytophthora pini, and Phytophthora cactorum were detected. In groundwater orchards, P. cinnamomi, P. pini, and P. cactorum were detected as well as Phytophthora rosacearum and Phytophthora nicotianae.

Our soil assays indicated that Phytophthora species are fairly common in orchard soils in the area served by the SEWD, with between a quarter and a half of orchards testing positive. Orchards irrigated with groundwater were equally likely to test positive for Phytophthora as orchards irrigated with surface water. It was also clear that Phytophthora species known to be aggressive pathogens of walnut and cherry were found in orchards regardless of what type of water is being used for irrigation. The source of Phytophthora inoculum in groundwater irrigated orchards is unknown, but we can hypothesize that Phytophthora may have been introduced to the soil during periodic river flooding or during historical use of surface water for irrigation. Historically, flood irrigation using surface water was widely used for orchard crops in the study region. Also, Phytophthora can be moved from one orchard to another on muddy or dirty farming equipment, and infested nursery stock can introduce Phytophthora to an orchard.



Results & DISCUSSION

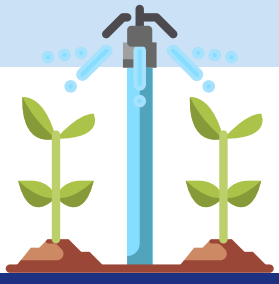
OBJECTIVE 3



Objective 3. Determine whether on-farm irrigation systems can deliver viable inoculum of Phytophthora from a surface waterway into an orchard

Baiting of irrigation water collected as it was applied to the orchard demonstrated that viable Phytophthora propagules were passing through the irrigation systems (Table 6). Of the 10 baiting trials in surface water irrigated orchards, nine detected viable Phytophthora in the water exiting irrigation emitters. There was no apparent effect of irrigation system (full-coverage sprinklers vs drip). No viable Phytophthora was detected in the irrigation water from the two groundwater irrigated orchards.

These results clearly indicate that infectious Phytophthora can move from a waterway into an orchard via the irrigation system. This was true with both drip irrigation and full-coverage sprinkler irrigation, and true with or without filtration by a sand media filter. We did not find enough orchards using microsprinklers to test that irrigation system type.



Recommendations

While the results of this study clearly show that there is Phytophthora in the SEWD waterways throughout the irrigation season, and that infectious Phytophthora can survive passage through irrigation systems into orchards, we did not find Phytophthora more frequently in soils of orchards irrigated with surface water than in those irrigated with groundwater. Surprisingly, we detected Phytophthora in up to half of the sampled orchards, though very few orchards showed symptoms of Phytophthora infection. These results indicate that in the SEWD, surface water irrigation by itself is not a large risk factor for an orchard developing Phytophthora infection. Instead, it is likely that good irrigation management practices are crucial for preventing Phytophthora infections in all orchards, regardless of the irrigation water source.

Phytophthora species rely on free water, or water-saturated soil, for significant spread and infection. Water-saturated conditions trigger the production of swimming spores that can sense, move toward, and infect plant tissue. The amount of time required to produce these swimming spores depends on the species of Phytophthora, but ranges from around 12 to 48 hours. This close association between water-saturated conditions and infective activity of Phytophthora species is the basis for recommending careful soil water management to minimize losses due to Phytophthora diseases. This is especially important given the significant proportion of Phytophthora-positive orchard soils in the SEWD. Irrigation recommendations include:

1) Keeping water away from the trunk and root crown. Plant trees on a berm. Move drip lines away from the tree as soon as possible after planting, with their final position at least 2 to 3 feet from the trunk. Use stream splitters on sprinklers to prevent trunk wetting.

2) Preventing prolonged soil water saturation by keeping irrigation sets short. In many orchards, 12 to 24 hours is a reasonable maximum run time, with irrigation frequency adjusted on several occasions over the growing season to meet but not exceed total orchard evapotranspiration demand.

3)Not exceeding the infiltration rate of your soil during irrigation. Proper irrigation system design helps to minimize the occurrence of standing water on the soil surface.

4)Not overirrigating trees. It is especially easy to overirrigate in the spring if you start irrigations too early.

Recommendations

CONTINUED

Another important strategy for reducing the chance for and impact of Phytophthora infection in an orchard is the use of resistant rootstocks. Table 7 summarizes the Phytophthora species found in this study which are pathogenic on walnut, cherry, or other crops grown in the area served by the SEWD, and Table 8 summarizes the information we have about rootstock resistance to Phytophthora. In many cases, rootstocks have only been systematically tested against one or two Phytophthora species. Briefly, RX1 (McGranahan et al., 2010; Browne et al, 2011) is a rootstock available for walnuts which has demonstrated resistance to both P. cinnamomi and P. pini and has been successfully used in many orchards. There is screening underway to find additional walnut rootstocks that are resistant to P. cinnamomi and P. pini as well as to the root lesion nematode and Agrobacterium tumefaciens, the cause of crown gall disease. It should be noted that Northern California black walnut and English walnut are both highly susceptible to Phytophthora infection. Among rootstocks for sweet cherry production, Mahaleb is highly susceptible to Phytophthora, but Colt and Mazzard offer some resistance to several species. Some work has been done using M2624 rootstock, which is resistant to Phytophthora but which requires an interstock for graft compatibility with cherry (Tersoglio & Setien, 2016), however this option is not widely used or easily available. While using a resistant rootstock offers protection from infection by Phytophthora species, it is important to realize that no known rootstock offers complete protection from soilborne disease problems. Resistant rootstocks should be used as part of an integrated approach, along with the water management practices detailed above, to protect orchards from Phytophthora.

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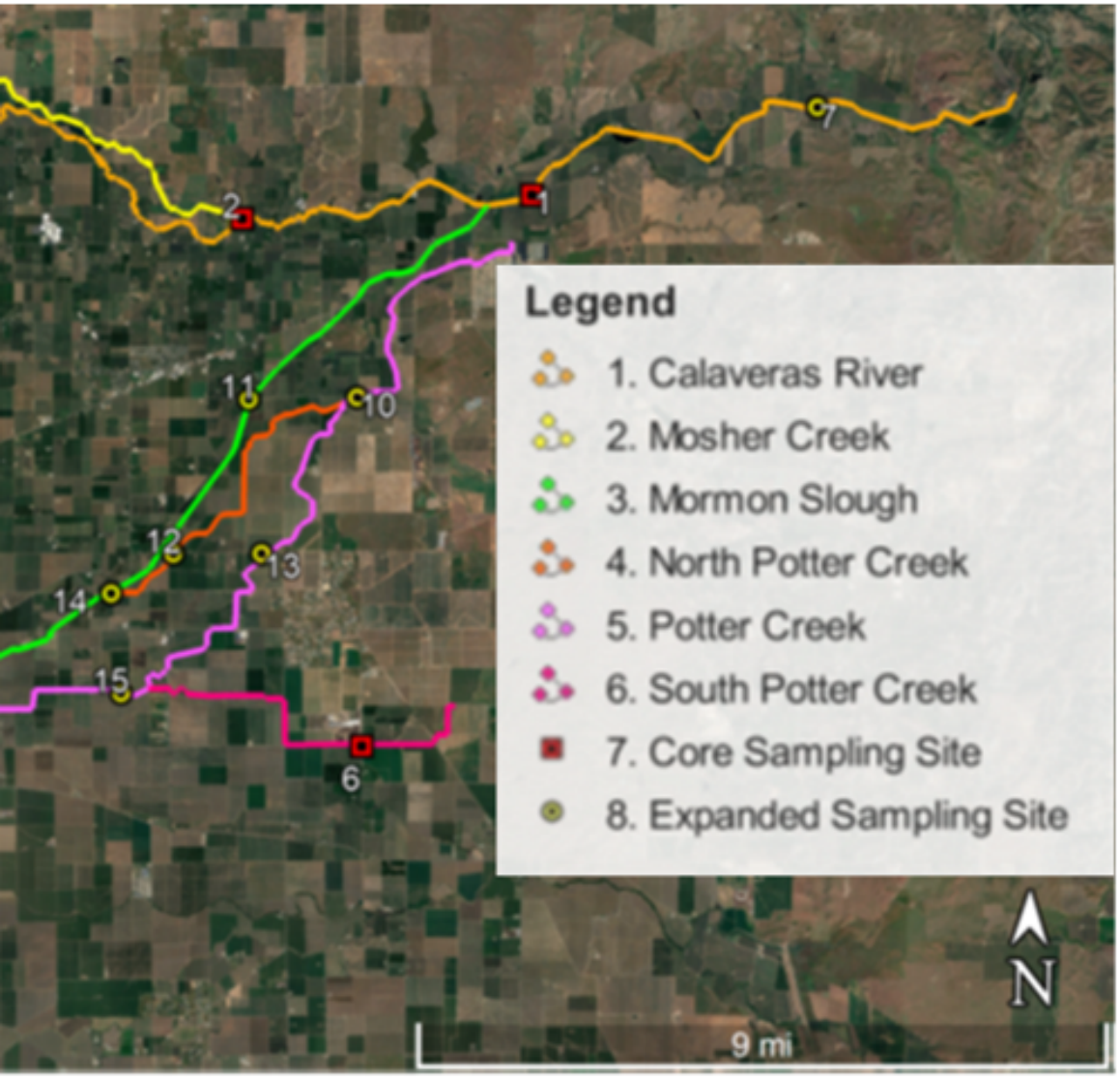
Figure 1

MAP OF WATERWAYS

MAP OF THE STOCKTON EAST WATER DISTRICT (SEWD), WITH SAMPLING SITES AND WATERWAYS INDICATED.

AND SAMPLING SITES



CORE SITES WERE SAMPLED JUNE, EARLY-JULY, MID-JULY, SEPTEMBER, AND OCTOBER. EXPANDED SITES WERE SAMPLED MID-JULY AND OCTOBER

Table 1

SUMMARY OF SAMPLING SITES & SAMPLING DATES

			Sampling date, 2022				
Site	Waterway	Type	4-Jun	3-Jul	15-Jul	14-Sep	11-Oct[a]
Site 1	Calaveras River	Core	*	*	*	*	*
Site 2	Calaveras River	Core	*	*	*	*	*
Site 3	Mosher Creek	Core	*	*	*	*	
Site 4	Calaveras River	Core	*	*	*	*	*
Site 5	Mormon Slough	Core	*	*	*	*	*
Site 6	South Potter Creek	Core	*	*	*	*	*
Site 7	Calaveras River	Expanded			*		*
Site 8	Mosher Creek	Expanded			*		*
Site 9	Calaveras River	Expanded			*		*
Site 10	Potter Creek	Expanded			*		*
Site 11	Mormon Slough	Expanded			*		*
Site 12	Potter Creek	Expanded			*		*
Site 13	Potter Creek	Expanded			*		*
Site 14	Mormon Slough	Expanded			*		*
Site 15	Potter Creek	Expanded			*		*

[a] Site 3 was dry at the October sampling, so no sample was collected.

Table 2

SUMMARY OF EMITTER BAIT SAMPLING

Date	Location[a]	Water source	Waterway	Emitter type	Filtration[b]
9/27/2021	1	Surface	Calaveras River	Sprinkler	None
9/27/2021	Intake A	Surface	Calaveras River	River	-
7/6/2022	2	Well	-	Sprinkler	None
7/6/2022	1	Surface	Calaveras River	Sprinkler	Sand media
7/6/2022	Intake A	Surface	Calaveras River	River	-
9/13/2022	1	Surface	Calaveras River	Sprinkler	Sand media
9/13/2022	3	Surface	Calaveras River	Drip	Sand media
9/13/2022	Intake A	Surface	Calaveras River	River	-
9/17/2022	2	Well	-	Sprinkler	None
9/30/2022	4	Surface	Potter Creek	Drip	None
9/30/2022	Intake B	Surface	Potter Creek	River	-
10/3/2022	1	Surface	Calaveras River	Sprinkler	Sand media
10/3/2022	3	Surface	Calaveras River	Drip	Sand media
10/3/2022	Intake A	Surface	Calaveras River	River	-
10/5/2022	4	Surface	Potter Creek	Drip	None
10/5/2022	Intake B	Surface	Potter Creek	River	-
10/11/2022	5	Well	-	Drip	None

[a] "Intake A" supplied water for both Location 1 and Location 3. "Intake B" supplied water for Location 4.

[b] A sand media filter was added to the irrigation system at Location 1 and Location 3 between the 2021 sampling and the subsequent sampling in 2022.

Table 3

SUMMARY OF PHYTOPHTHORA DETECTIONS AT EXPANDED WATERWAY SITES, BASED ON PCR AND PACBIO SEQUENCING[a]

Known Importance	Species	Calaveras River												
		Site 1					Site 2							
		6/4	7/3	7/15	9/14	10/11	6/4	7/3	7/15	9/14	10/11	6/4	7/3	
Important walnut or cherry pathogens	*P.cactorum*	-	*	*	-	-	*	*	*	*	-	-	-	
	P.cambivora	-	-	-	-	-	-	*	-	-	-	-	-	
	P.citricola/pini/ plurivora	*	-	-	-	-	*	*	-	-	-	*	*	
	P.citrophthora	-	-	*	-	-	*	*	*	*	-	*	-	
	P.pini	-	-	-	-	-	-	*	-	-	-	*	-	
Infect walnut or cherry, but not widely important on those crops	*P.chlamydospora*	-	-	*	-	-	-	*	-	*	-	*	*	
	P.gonapodyides	-	*	*	-	-	*	*	*	*	-	*	-	
	P.rosacearum	-	*	-	-	-	*	*	*	*	-	*	-	
	P.taxon walnut	-	-	-	-	-	*	*	-	*	-	*	-	
Not known to infect walnut or cherry, but important pathogens on other crops in the SEWD	*P.acerina*	*	*	*	*	-	*	-	-	-	-	-	-	
	P.capsici	-	-	-	-	-	-	-	-	-	-	-	*	
	P.mediterranea	-	-	-	-	-	*	*	*	-	-	*	*	
	P.niederhauserii	-	-	*	-	-	-	*	-	-	-	*	*	
	P.crassamura	-	-	-	-	-	-	-	-	-	-	-	-	
Not known to cause disease on crops common in the SEWD	*P.europaea*	-	-	-	-	-	-	-	-	-	-	-	-	
	P.parsiana	-	-	-	-	-	-	*	-	-	-	-	-	
	P.bishii	-	-	-	-	-	-	*	-	-	-	-	-	
	P.cf. sp. bitahaiensis	-	-	-	-	-	-	-	-	-	-	-	-	
	P.cf. sp. sylvatica1	-	-	-	-	-	*	*	-	-	-	*	*	
	P.lacustris	*	*	*	*	-	*	*	*	*	-	*	*	
	P.mississippiae	-	*	*	-	-	*	*	*	*	-	*	-	
	P.personensis/ inundata	-	-	-	-	-	-	-	-	-	-	-	-	
	P.riparia	*	*	*	*	-	*	*	*	*	-	*	*	
	P.sp. oaksoil/ bilorbang	-	-	-	-	-	-	-	-	-	-	-	-	
	P.taxon raspberry	-	-	-	-	-	-	-	-	-	-	-	-	
	P.unk sp	*	*	-	-	-	*	*	*	*	-	*	-	

a Asterisks (*) indicate a positive detection of a Phytophthora sp. in a given sample, whereas "-" symbols indicate no detection of that species. Detections were based on ITS amplification and PacBio sequencing as described in methods.

				Mosher Creek					Mormon Slough					South Potter Creek				
Site 4				Site 3					Site 5					Site 6				
3	7/15	9/14	10/11	6/4	7/3	7/15	9/14	10/11	6/4	7/3	7/15	9/14	10/11	6/4	7/3	7/15	9/14	10/11
	*	-	-	-	-	-	-		-	-	-	-	-	-	-	-	*	-
	-	*	-	-	-	-	-	not collected	-	-	-	-	-	-	-	-	-	-
*	*	-	-	*	-	-	-		-	-	-	-	-	-	-	-	-	-
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Table 4

SUMMARY OF PHYTOPHTHORA DETECTIONS AT EXPANDED WATERWAY SITES, BASED ON PCR AND PACBIO SEQUENCING[a]

		15-July Sampling												
		Calaveras River					Mosher Creek		Mormon Slough			Potter Creek		
Known Importance	Species	Site7	Site1	Site2	Site9	Site4	Site8	Site3	Site11	Site14	Site5	Site10	Site12	Site13
Important walnut or cherry pathogens	*P.cactorum*	*	*	*	-	*	-	-	-	-	-	*	-	-
	P.citricola/pini/ plurivora	-	-	-	*	*	-	-	-	-	-	*	-	*
	P.citrophthora	*	*	*	-	-	-	*	-	-	-	-	-	-
	P.plurivora	-	-	-	-	-	-	-	-	-	-	*	-	-
Infect walnut or cherry, but not widely important on those crops	*P.chlamydospora*	*	*	-	-	*	*	-	-	-	-	*	-	*
	P.gonapodyides	*	*	*	-	*	*	*	-	-	-	*	-	*
	P.rosacearum	-	-	*	-	-	-	-	-	-	-	-	-	-
	P.taxon walnut	-	-	-	-	-	-	-	-	-	-	*	-	-
Not known to infect walnut or cherry, but important pathogens on other crops in the SEWD	*P.acerina*	-	*	-	-	-	-	-	-	-	-	-	-	-
	P.capsici	-	-	-	-	-	-	-	-	-	-	*	-	-
	P.mediterranea	-	-	*	-	*	-	*	-	-	-	*	-	-
	P.niederhauserii	*	*	-	-	-	-	-	-	-	-	-	-	-
Not known to cause disease on crops common in the SEWD	*P.europaea*	-	-	-	-	-	*	*	-	-	-	-	-	-
	P.bishii	-	-	-	-	*	-	-	-	-	-	-	-	-
	P.cf. sp. sylvatica1	-	-	-	-	-	-	*	-	-	-	-	-	-
	P.lacustris	*	*	*	-	*	*	*	-	-	-	*	-	*
	P.mississippiae	*	*	*	*	*	*	*	-	-	-	*	-	*
	P.personensis/ inundata	-	-	-	-	-	*	-	-	-	-	-	-	-
	P.riparia	*	*	*	*	*	*	*	-	-	-	*	-	*
	P.sp. oaksoil/ bilorbang	-	-	-	-	-	-	-	-	-	-	*	-	-
	P.x multiformis	*	-	-	-	-	-	-	-	-	-	-	-	-
	P.unk sp	*	-	*	*	*	*	*	-	-	-	*	-	*

Asterisks (*) indicate a positive detection of a Phytophthora sp. in a given sample, whereas "-" symbols indicate no detection of that species. Detections were based on ITS amplification and PacBio sequencing as described in methods.

11-October Sampling																	
	S. Potter Creek	Calaveras River					Mosher Creek		Mormon Slough			Potter Creek				S. Potter Creek	
Site15	Site6	Site7	Site1	Site2	Site9	Site4	Site8	Site3	Site11	Site14	Site5	Site10	Site12	Site13	Site15	Site6	
-	-	-	-	-	-	-	-	not collected	-	-	-	-	-	-	-	-	
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Table 5

SUMMARY OF TOTAL PHYTOPHTHORA DETECTIONS FROM ORCHARD SOILS USING CULTURE-BASED & CULTURE-INDEPENDENT METHODS

	Least stringent (500 Reads)		Moderately stringent (1000 Reads)		Most stringent (10,000 Reads)	
	Ground-water	Surface Water	Ground-water	Surface Water	Ground-water	Surface Water
No. of orchards sampled:	20	20	20	20	20	20
No. of orchards positive for *Phytophthora*:	9	8	7	5	5	5
Chi Squared test (for ground vs. surface water):	$P = 1.0$		$P = 0.6$		$P = 1.0$	

Table 6

SUMMARY OF PHYTOPHTHORA DETECTIONS FROM IRRIGATION EMITTERS & IRRIGATION INTAKE POINTS

Irrigation water source	No. of times sampled		No. *Phytophthora* positive samples	
	Sprinkler	Drip	Sprinkler	Drip
Groundwater[a]	2	1	0	0
Surface Water[a]	6	4	5	4
River[b]	6		6	

[a] Water collected after discharge from irrigation emitters.

[b] Water collected directly from the waterways near the pump intake for the "Surface Water" irrigation systems.

Table 7

GENERAL CHARACTERIZATION OF CROP DISEASE RISKS PRESENTED BY PHYTOPHTHORA SPECIES FOUND IN SEWD SURFACE WATER OR IN ORCHARD SOILS IN AREAS SERVED BY THE SEWD

Category	*Phytophthora* species	Known host status of selected crops grown widely in SEWD
Known as important pathogens of orchard crops grown in SEWD	*P. acerina*	Almond
	P. cinnamomi	Almond, cherry, walnut
	P. crassamura	Almond
	P. nicotianae	Almond, cherry, walnut, pepper, tomato
	P. cactorum	Almond, cherry, walnut
	P. cambivora	Almond, cherry
	P. citricola/pini/ plurivora	Almond, cherry, walnut
	P. citrophthora	Cherry, walnut
	P. mediterranea	Almond, pistachio
	P. niederhauserii	Almond, pistachio
	P. pini	Almond, walnut
	P. plurivora	Cherry, walnut
Known to infect some SEWD orchard crops, but not known as important pathogens on them	*P. chlamydospora*	Almond, cherry
	P. gonapodyides	Walnut
	P. rosacearum	Cherry
	P. taxon walnut	Almond, pistachio, walnut
Known only as important pathogen of vegetable crops grown in the SEWD	*P. capsici*	Pepper, tomato

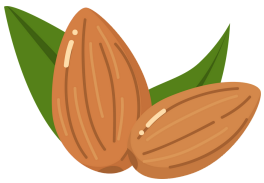
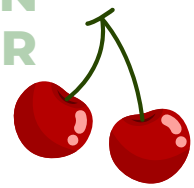


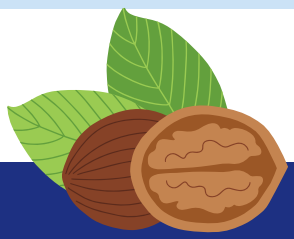
Table 8

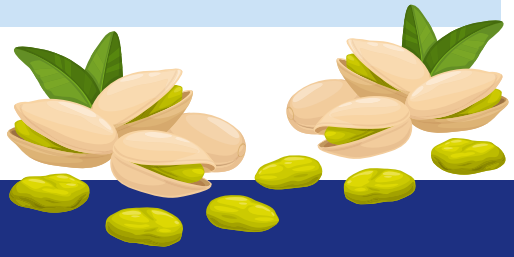
RESISTANCE TO PHYTOPHTHORA SPECIES AMONG COMMERCIALLY AVAILABLE ROOTSTOCKS FOR FRUIT AND NUT CROPS COMMONLY GROWN ON LAND SERVED BY THE STOCKTON EAST WATER DISTRICT [a]



Orchard crop	Comments of rootstock resistance to Phytophthora species
Almond	Nemaguard peach rootstock has shown greater resistance to several *Phytophthora* species than clonal hybrid almond rootstocks that include almond as a parent (e.g., Hansen 536, Titan, BB 106, Viking). Marianna 2624 rootstock, which is not graft compatible with Nonpareil almond but can be used with several other cultivars, is highly resistant to several *Phytophthora* species.
Cherry	Mazzard and Colt rootstocks have shown greater resistance to *Phytophthora* species than Mahaleb rootstock, which is highly susceptible to several species of *Phytophthora*.
Pistachio	Pistachio rootstocks are not regarded as highly susceptible to *Phytophthora* species, but testing of the resistance is underway
Walnut	RX1 rootstock has shown a high level of resistance to *P. cinnamomi*, the most aggressive species of *Phytophthora* on walnut. In contrast, Northern California black seedling and English walnut seedling rootstocks are highly susceptible to *Phytophthora* species; while Paradox hybrid seedlings, clonal Vlach Paradox hybrid, and clonal VX211 Paradox hybrid rootstock are moderately susceptible to several *Phytophthora* species.

[a]For updates and additional details, see: https://fruitsandnuts.ucdavis.edu/ and search by crop for rootstock information. For example, sources of information include: the Almond Rootstock Database and Almond Rootstock Chart available under the almond tab; the Walnut Rootstock and Scion Selection section under the walnut tab, and the Cherry Scion and Rootstock Selection section under the cherry tab of the Fruit and Nut Research Information Center. Also, it is advisable to check with your UC Farm Advisor for recent updates on rootstocks for specific crops.





END OF REPORT

STOCKTON EAST WATER DISTRICT

PROVIDING SERVICE SINCE 1948

1948 75 2023

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