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Genetic and phenotypic variation of *Phytophthora crassamura* isolates from California nurseries and restoration sites

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ABSTRACT

Phenotypic and sequence data were used to characterize 28 isolates resembling *Phytophthora megasperma* from 14 host species in 2 plant production facilities and 10 restoration sites across the San Francisco Bay Area (California; USA). Size of the oogonia and DNA sequences (nuclear internal transcribed spacer (ITS) and mitochondrial cytochrome c oxidase subunit 1 (COX 1)) were compared, and sensitivity to mefenoxam and pathogenicity were measured. Based on ITS 61 % of isolates matched ex-type sequences of *Phytophthora crassamura* from Italy, and the remainder matched or were close to the *P. megasperma* ex-type. However, all California *P. crassamura* genotypes belonged to four unique COX 1 haplotype lineages isolated from both nurseries and restoration sites. Although lineages were sensitive to mefenoxam, a significant difference in sensitivity was identified, and all continued growth in-vitro. These results suggested previous mefenoxam exposure in plant production facilities resulting in tolerance. In conclusion, all evidence pointed to a nursery origin of novel *P. crassamura* lineages found in California restoration sites. In this study, COX 1 sequences and oogonia size provided information relevant to identify geographic and evolutionary intraspecific variation within *P. crassamura*, and was additionally used to track the spread of this species from nurseries into wildlands.

1. Introduction

This study investigates the genetic and morphological variation among a set of '*Phytophthora megasperma*-like' isolates belonging to the *P. megasperma* species complex and obtained from a number of California (USA) plant production facilities and restoration sites around the San Francisco Bay Area. The taxonomic focus of this study was further motivated by the recent description of a new species within the *megasperma* complex, namely *Phytophthora crassamura*, causing significant plant mortality in the Sardinian archipelago of La Maddalena (Scanu et al., 2015), a region characterized by a climate similar to that of California.

The *P. megasperma* species complex has long been the focus of significant research, first using morphological approaches to differentiate taxa included in the complex, then using DNA sequence information. Dreschler (1931) first described *P. megasperma* sensu stricto as a clearly defined species with a geographic range limited to the District of Columbia, possibly extending as far west as Wisconsin, and only from a single host, hollyhock (*Alcea rosea*). However, soon after the initial description, other authors identified *P. megasperma* as a species complex with a more varied morphology, broader host range, and a much wider global distribution than initially published (Tompkins et al., 1936; Erwin, 1954, 1965; Hildebrand, 1959). The size of oogonia was later identified as a diagnostically significant trait within the newly recognized species complex, and variants with smaller oogonia were officially described (Waterhouse, 1963; Faris et al., 1989). Only recently, have the morphologically relevant variants been given the status of species distinct from *P. megasperma* sensu stricto, thanks to the support of DNA sequence information (Tyler, 2006; Hansen et al., 2009; Scanu et al., 2015). For instance, some *Phytophthora* isolates deposited as *P. megasperma* in Japanese culture collections were described as belonging to the species *P. lilii*, occupying a completely

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different sub-generic clade within the genus (Rahman et al., 2014, 2015), clade 11 (Jung et al., 2017; Yang et al., 2017).

The focus on *Phytophthora* species present in California restoration sites is relatively new, and has stemmed from the recent discovery that restored sites were infested by these potentially serious plant pathogens (Rooney-Latham and Blomquist, 2014; Rooney-Latham et al., 2015; Garbelotto et al., 2018a, b). Notwithstanding these findings, it has not been definitively demonstrated that *Phytophthora* genotypes in restored sites across California may have originated from infected plant stock used in restoration projects (Garbelotto et al., 2018a, b). Restoration efforts are complex, costly, involve many stakeholders, and -above all-are meant to restore the integrity of disturbed habitats by reversing land degradation, increasing the resilience of biodiversity, and delivering important ecosystem services (Wortley et al., 2013). If indeed plant stock infested by plant pathogens including *Phytophthora* species is used in restoration projects, then such complex and costly projects would potentially further damage disturbed ecosystems, rather than restoring their integrity. Worse, some of these pathogens may successfully spread to wildlands outside the boundaries of restoration sites, causing potentially large-scale plant disease outbreaks with detrimental effects on the environment, similar to what happened with *Phytophthora alni* across southern Britain especially in the southeast of England (Gibbs et al., 1999). Thus, the second aim of this study was to further investigate the connectivity between plant production facilities and restoration sites by assessing whether the same pathogens could be found in both.

In order to support the hypothesis that *Phytophthora* genotypes found in restoration sites come from plant production facilities providing plant stock for restoration, we also studied variation in sensitivity to the fungicide mefenoxam (Subdue MAXX; Syngenta, Greensboro, NC) across a sample of genotypes, both from restorations and plant production facilities. Mefenoxam is a systemic phenylamide fungicide used since 1978 for control of *Phytophthora* species diseases. It is probably the best fungicide for testing variability of sensitivity due to its long history of use. The phenomenon of variable sensitivity to mefenoxam is well documented in several oomycete plant pathogen species, including *Phytophthora* species, on a range of crops worldwide (Gisi and Cohen, 1996; Gisi and Sierotzki, 2008). Although exposed individuals do not always develop resistance, the presence of varying degrees of sensitivity across subpopulations is indicative of a history of exposure to mefenoxam that must have occurred either in an agricultural or a plant production setting, given that this chemical is seldom if ever used in natural settings. On the other hand, the complete and permanent arrest of growth of isolates following exposure would, therefore, indicate wild-type genotypes. In this study, isolates from wildlands were examined for a history of exposure indicated by variable sensitivity and survival following exposure.

Finally, even if the confirmed source of *Phytophthora* infestations in restorations were plant stock from infested California plant production, the question would remain on whether such *Phytophthora* genotypes cause significant disease. The final aim of this study was to show that a significant disease leading to widespread mortality of California sycamore (*Platanus racemosa*) saplings planted in a restoration site in Alameda County was indeed caused by a specific genotype belonging to the *P. megasperma* species complex, and found in both the disease site and in plant production facilities.

2. Methods

2.1. Investigated sites

Study sites and nurseries containing diseased plants were located across the San Francisco Bay Area (California, USA) in Santa Clara, Alameda, San Mateo, Marin and San Francisco Counties (Table 1). A few of the *P. crassamura* isolates from nurseries have been published in a study on the efficacy of best management practices (Sims et al., 2018). Samples consisted of soil and roots samples, or of plant stems. One to two liters of each sample, consisting of both soil and roots, were taken using sterilized shovels or spades. The organic layer was scraped off, and soil was collected including roots, up to 60 cm in depth, depending on root depth and accessibility, but was generally in the upper 25 cm depth. In the case of plant stem samples, the entire plant was uprooted and placed in plastic with a moist cloth (to avoid drying out the sample), in cold storage (5 °C) for up to 48 h until a stem portion was excised for pathogen isolation.

2.2. Baiting and stem isolation methods

Phytophthora isolation from soil samples was attempted through baiting (Erwin and Ribeiro, 1996), while isolation from symptomatic plant tissue was made through direct plating of symptomatic plant tissue.

Baiting was done using the following baits: an entire fruit or fruit pieces of *Pyrus communis* (pear) of the D'anjou variety (McIntosh, 1964), entire leaves or leaf pieces of *Rhododendron* 'Cunningham's White' (Sims et al., 2015), and *Origanum vulgare* (oregano) leaf and stem pieces used together. Baiting conditions were as follows: volume of bags was 1–2 L, amount of water was about 2 cm above the soil line, the temperature during baiting was about 72 °C, and time of baiting was 5 d. In the absence of any typical necrotic lesions on baits after the first baiting process, a double-baiting procedure was used. Such procedure involved drying out and rewetting the sample (Jeffers and Aldwinkle, 1987). At the end of the single or double-baiting process, bait pieces displaying typical necrotic lesions were submerged in selective ½ VARP + medium (V8 based agar amended with 10 ppm Pimaricin, 200 ppm Ampicillin trihydrate, 10 ppm Rifampicin, 15 ppm Benomyl and 25 ppm Hymexazol [97 %]) contained in 100 mm × 15 mm Petri dishes.

Direct *Phytophthora* isolations were performed only for plants showing typical canker-like symptoms. Direct isolations were performed by excising stem pieces that included both healthy and diseased stem tissue, by gently scraping off the outer bark with a scalpel, and by plating inner bark and cambium pieces at the conjunction of healthy and diseased tissue directly onto a selective ½ VARP medium (same as above except no Hymexazol).

2.3. DNA sequencing

DNA sequencing and haplotyping was done as described in previous works (Sims et al., 2015), but with minor revisions. The ribosomal region spanning the internal transcribed spacer (i.e., ITS; ITS1-5.8S-ITS2) was amplified using primers DC6 (Cooke et al., 2000) and ITS4 (White et al., 1990), while the mitochondrial region spanning the cytochrome oxidase c subunit 1 (COX 1) gene was amplified using primers FM84 (Martin and Tooley, 2003) and FM55 (Martin, 2000). Differently from Sims et al. (2015), GoTaq® DNA Polymerase with 5X Green GoTaq® Reaction Buffer (Promega) were used in PCR reactions, and the amplification conditions for COX 1 were as detailed by Scanu et al. (2015). PCR products were visualized in 1.5 % agarose gels (SuperPure™ Agarose LE) with lithium borate as an electrophoresis buffer (LB®, Faster Better Media LLC) and GelRed (2.5 ml per 50 ml gel). The gel was run for 15 min while submerged in the buffer and then visualized using Trans UV light and a BioRad® camera. PCR products were then prepped for sequencing (same primers as for amplification), and submitted for PCR clean-up and Sanger sequencing (University of California, Berkeley, DNA Sequencing Facility).

Table 1
Phytophthora crassamura and *P. megasperma* isolates used in this study. References used in the phylogenetic analysis are also included.

Identity	Collection No.	Host	Sample	Year of isolation	Location		haplotype	GenBank Accessions	
					County/location	R = restoration N = nursery; other		ITS	Cox1
P. crassamura	TB_144[b,c]	*Heteromeles arbutifolia*	Root and soil	2015	Santa Clara	R	i	MG707798	MH455229
P. crassamura	TB_449[b,c]	*Quercus agrifolia*	Root and soil	2015	Santa Clara	R	i	–	–
P. crassamura	WS_C85[c,d,f]	*Platanus racemosa*	Stem canker	2015	Alameda	R	i	MH443077	MH455222
P. crassamura	WS_C87[c,d,f]	*Platanus racemosa*	Root and soil	2015	Alameda	R	i	MH443078	MH455218
P. crassamura	WS_C91[c,d]	*Platanus racemosa*	Stem canker	2015	Alameda	R	i	–	–
P. crassamura	WS_C79[c]	no recognizable host	Contractor plant waste: Soil and plant debris	2016	Marin	stock pile for trail use	i	–	–
P. crassamura	WS_C96[c]	no recognizable host	nursery debris	2016	Marin	N	i	MH443079	MH455224
P. crassamura	WS_C82[c,e,f]	*Juncus effusus*	Root and soil	2015	Marin	N	i	MH443076	MH455225
P. crassamura	WS_E47[c,f]	*Alnus rubra*	Root and soil	2015	Marin	N	i	MH443082	MH455227
P. crassamura	WS_D41[c]	*Eriophyllum staechadifolium*	Root and soil	2015	San Mateo	R	i	MH443080	MH455226
P. crassamura	WS_E11[c,e]	*Rosa species*	Root and soil	2016	San Francisco	WUI[g]	i	MH443081	MH455223
P. crassamura	TB_586[b,c]	*Sambucus mexicana*	Root and soil	2016	Santa Clara	R	iia	–	–
P. crassamura	TB_557[b,c]	*Rosa californica*	Root and soil	2015	Santa Clara	R	iia	MH443073	MH455228
P. crassamura	TB_464[b,c]	*Salix* sp.	Root and soil	2015	Santa Clara	R	iia	–	–
P. crassamura	TB_549[b,c]	*Diplacus aurantiacus* and *Artemisia californica*	Root and soil	2015	Santa Clara	R	iia	–	–
P. crassamura	WS_C76[c,e,f]	*Pinus torreyana*	Root and soil	2016	San Francisco	N	iib	MH443075	MH455216
P. crassamura	WS_F1[c,e,f]	*Frangula californica*	Root and soil	2016	San Mateo	R	iib	MH443084	MH455219
P. crassamura	WS_J43[c,f]	*Frangula californica*	Root and soil	2016	San Mateo	R	iib	MH443085	MH455221
P. crassamura	WS_C67[c,f]	no recognizable host	Contractor plant waste: Soil and plant debris	2016	Marin	stock pile for trail use	iib	MH443074	MH455220
P. crassamura	TB_539[b,c]	*Artemisia douglasiana*	Root and soil	2015	Santa Clara	R	iii	–	–
P. crassamura	TB_442[b,c]	*Artemisia douglasiana*	Root and soil	2015	Santa Clara	R	iii	–	MH455230
P. crassamura	TB_413[b,c]	*Artemisia douglasiana*	Root and soil	2015	Santa Clara	R	iii	MH443072	MH455217
P. megasperma[a]	WS_L52[c,f]	*Diplacus aurantiacus*	Stem canker	2017	Marin	R		MH443086	–
P. megasperma[a]	WS_L67[c,f]	*Diplacus aurantiacus*	Stem canker	2017	Marin	R		MH443087	–
P. megasperma[a]	WS_K14[c,f]	*Diplacus aurantiacus*	Stem canker	2017	Marin	R		–	–
P. megasperma[a]	WS_M3[c,f]	*Diplacus aurantiacus*	Root and soil	2017	Marin	R		MH443088	–
P. megasperma	WS_E66[c,e]	Weeds: *Rubus* species and *Hedera helix*	Root and soil	2016	San Francisco	WUI[g]	type	MH443083	MH455231
P. megasperma	WS_C95[c,e]	no recognizable host	Contractor plant waste: Soil and plant debris	2016	Marin	stock pile for trail use	type	–	–
References for Phylogenetic analysis					Country				
P. crassamura	PH138	*Juniperus phoenicea*	root and soil	2012	Italy	wetland	type	**KP863493**	**KP863485**
P. megapserma	CBS 402.72	*Alcea rosea*	root rot	1931	USA	planting	type	**HQ643275**	**KP863479**
P. crassamura	PH094	*Picea abies*	collar lesion	2011	Italy	N	type	KP863492[h]	KP863482
P. crassamura	PH170	*J. phoenicea*	root and soil	2012	Italy	wetland	type	KP863494[h]	KP863483
P. crassamura	PH171	*J. phoenicea*	root and soil	2013	Italy	forest	type	KP863495[h]	KP863484
P. crassamura	DDS3432	*Banksia species*	soil	1992	Australia		iic	KP863496	HQ012867
P. crassamura	VHS17183	*Xanthorrhoea platyphylla*	soil	2007	Australia		iid	KP863497	HQ012868
P. megapserma	PH192	*Castanea sativa*	root and soil	2013	Italy	R		KP863498	KP863481
P. megapserma	PH178	*Castanea sativa*	root and soil	2013	Italy	R		KP863499	KP863480

[a] *P. megasperma* complex isolates (with SNP's and heterozygous alleles).
[b] DNA from UC Davis study no voucher.
[c] Included in phylogenies.
[d] Used in pathogenicity test.
[e] Used in morphology test.
[f] Used in fungicide test.
[g] Wildland-urban interface.
[h] Used in phylogenetic analysis but removed from phylogram. Ex-type accessions are boldfaced.

2.4. Phylogenetic evaluation of P. crassamura for haplotypes

The Bioedit program (Hall, 1999) was used to trim all the sequences to the same size for comparisons. Phylogenetic analysis was performed with the program MrBayes 3.2 (Huelsenbeck and Ronquist, 2001). Evolutionary models were set to the General Time Reversible with a proportion of invariable sites and gamma-shaped distribution ($GTR + I + \Gamma$). The analysis was run for one million generations for both COX 1 and ITS as this was adequate to reach convergence based on a set of measurable criteria. The model was checked for convergence in three ways: (1) with the average standard deviation of split frequencies confirmed to be below 0.01 and approaching zero (COX 1 = 0.007, ITS = 0.008), (2) with the Potential Scale Reduction Factor (PSRF) value (Gelman and Rubin, 1992; Ronquist et al., 2011) confirmed as approaching 1 (COX 1 PSRF average = 1.000 and ITS PSRF average = 1.000), (3) and by establishing the convergence plots displayed no discernable pattern.

The *P. megasperma* ex-type sequences for both the ITS and COX 1 phylogenies were used as outgroups to the *P. crassamura* ingroup. This is because *P. crassamura* groups in a well-supported sister clade to that including isolates of *P. megasperma* (Scanu et al., 2015), and *P. megasperma* is not considered part of the *P. crassamura* ingroup as it is genetically more than twice as distant than any member of the ingroup. It is well known that for resolving the evolutionary relationships of a clade within a genus, the most appropriate outgroup would be a member of the sister clade (Baum and Smith, 2013). *Phytophthora gonapodyides* and *Phytophthora riparia* were also tested as potential outgroup candidates, but due to no discernable advantages, their use was dropped (data not shown). *P. crassamura* sequences including those derived from the ex-type were also included in phylogenies (Table 1). Identical sequences were assumed to represent the same haplotype.

2.5. Oogonia dimension comparison of P. megasperma and P. crassamura

The two *P. crassamura* haplotypes found both in California wildlands and nurseries, and *P. megasperma* sensu stricto, were morphologically compared by examining the size of the oogonia after oospore formation. Photographs of these were taken at 400× magnification using a Nikon Eclipse E400 compound microscope (Japan) and a Nikon D90 camera. A total of six isolates were examined: two isolates for each of the two *P. crassamura* haplotypes and two isolates for *P. megasperma*. Forty mature and normal oogonia from each isolate (aborted or damaged oogonia were excluded) were measured twice in diameter along two orthogonal directions.

2.6. Statistical evaluation of oogonia size

The statistical program R (R core group, 2018) was used to compare oogonia sizes of the two California haplotypes to one another and the sizes of oogonia of *P. megasperma* sensu stricto. An analysis of variance was performed, to check differences in oogonia size (estimated average mean diameter) across the three haplotypes (evaluated using f-statistics). Normality and homogeneity of variance were checked for adequacy before reporting results.

2.7. Tests comparing the sensitivity of Phytophthora genotypes to mefenoxam

An experiment was conducted to characterize the sensitivity to mefenoxam of a total of 12 “*P. megasperma*-like” isolates and three replicates of each isolate (Table 1). The experiment consisted of measurements of colonies growing on medium amended with six different concentrations of mefenoxam (0, 0.1, 1, 5, 10, and 100 μg/ml). The base medium for the *in vitro* fungicide evaluation was filter clarified 10 %V8 (10-V8A) agar. Mefenoxam was diluted in sterile distilled water and added to the autoclaved medium cooled to 40 °C. Mycelium plugs (5 mm in diameter) were cut from the margin of actively growing 5-day-old colonies. One mycelial plug was placed in the center of a 10-cm diameter Petri dish in contact with the medium and then incubated at 18 °C. Measurements were taken at 7 d as follows: colony diameters from two perpendicular directions were measured and averaged, and the averaged mean calculated from the three replicates of each isolate at each concentration. The relative mycelium growth was calculated by dividing the averaged mean colony diameter for each isolate in amended dishes by that of the non-amended control dishes. Isolates were scored at each concentration as sensitive ($\leq$10 % growth), intermediately sensitive (11–40 % of control), and resistant (growth $\geq$40 % of control) (Hu et al., 2010). Isolates that were intermediately sensitive at the highest concentration (100 μg/ml) were considered tolerant.

The Dose Response Curve (drc) package (Ritz et al., 2015) in the R program (R Core Team, 2018) was used to determine EC50 values. An analysis was done using a single concentration-response log–logistic curve with four parameters to model the data (Inderjit et al., 2002). The function ‘mselect’ then provided a summary of fit statistics for several different models (1 log normal, 1 additional log-logistic, and 2 Weibull) fitted to the same data. The resulting Akaike Information Criterion (AIC) fit statistics were then used with the ‘maED’ function to give weighted estimates and confidence intervals for the best fits (Kang et al., 2000). If the log–logistic curve and other curves did not fit, only one Weibull curve was used, as this was the only model that fitted the data. In these cases, the Delta Method, i.e., the calculation of approximate standard errors for derived parameter estimates, was used to determine confidence intervals (Weisberg, 2005).

2.8. Pathogenicity test

Three-year-old *P. racemosa* (California sycamore) plants were randomly assigned to one of four inoculation treatments, each consisting of one of three isolates that had been isolated from different plants in an Alameda County restoration site (Table 1), or of a mock control inoculation consisting of a sterile plug of agar. All inoculated plants were incubated in an indoor quarantine-level grow room at 21 °C for 30 d. Lesion size (canker area in mm^2) was estimated, and the mean canker area and standard deviation for each of the three isolates and the control were calculated and graphed. A model was constructed to evaluate the difference in the canker size from the *P. crassamura* treated trees compared to the control treated ones after accounting for the random effects of each tree using a linear mixed effect model and the package *nlme* (Pinheiro et al., 2018). The modeled treatment effect was evaluated using the f-statistic, and differences between isolates with the t-statistic.

3. Results

3.1. Phytophthora isolations

Overall, *P. crassamura* was widespread having been isolated 55 times, and other *P. megasperma* genotypes were less common having been isolated only 17 times (see Table 1 for information on pathogen isolates and hosts). The number of *P. crassamura* isolates collected by county were as follows: Alameda, 3; Marin, 23; Monterey, 1; San Francisco, 6; San Mateo, 10; and Santa Clara, 12. The numbers of other *P. megasperma* isolates collected by county were as follows: Alameda, 0; Marin, 10; Monterey, 0; San Francisco, 4; San Mateo, 1; and Santa Clara, 2. A total of 8 *P. crassamura* isolates were collected from heavily-trafficked wildlands; 7 were collected from nurseries; 22 from restorations; and 18 from trail soil. The number of *P. megasperma* isolates collected from disturbed wildlands was 4; 1 from nurseries; 10 from restorations, and 2 from trail soil.

3.2. Santa Clara County

P. crassamura was collected in 6/31 sites and *P. megasperma* from 1/31. *P. crassamura* was baited from soil associated with *Artemisia douglasiana*, *Artemisia californica*, *Diplacus aurantiacus*, *Rosa californica*, *Salix* species, *Heteromeles arbutifolia*, *Quercus agrifolia*, and *Sambucus mexicana*. *P. megasperma*, not including *P. crassamura*, was associated with *D. aurantiacus* and *Frangula californica*. *P. crassamura* was the second most commonly isolated *Phytophthora* species after *Phytophthora cactorum*.

3.3. Alameda County

From the one restoration site examined, only *P. crassamura* was isolated from three of six California sycamore plants that were tested. One positive was from the stem, and double baiting from the roots was necessary to obtain the other two positives (Fig. 1). Disease symptoms on California sycamores at this site included aerial cankers that were girdling the stem and were not extending into the root collar or the roots. Stem tissue above cankers was necrotic.

3.4. San Mateo County

Plants from two of three restoration sites, yielded positive *P. crassamura* isolations. One site was a planted restoration, and the other two were disturbed wildland areas. *P. crassamura* was isolated from three of ten *D. aurantiacus* plants and from three of ten *F. californica* plants in the planted restoration site, as well as from one of the disturbed wildland sites, where four of ten *F. californica* and none of the five *D. aurantiacus* were positive.

3.5. Marin County

Four restoration sites were evaluated in Marin, and two contained positives. At one site, symptoms included cankers that extended from the root systems into the root collar and stem (Fig. 2). *P. megasperma* was isolated from the stem of 4 symptomatic out of 10 *D. aurantiacus* plants, 3 of which also had visible aerial stem cankers. The roots of one additional and symptomatic *D. aurantiacus* plant were positive for *P. crassamura*. Also at this site three *F. californica* plants also had symptoms, and one was positive for *P. megasperma*. From the other site, a total of 18 stockpiled trail soil samples were positive for either *P. crassamura* or *P. megasperma*.

3.6. Grouping isolates based on ITS

The aligned ITS sequences were each 669 characters long with no indels. Isolates of *P. crassamura* grouped with the ex-type *P. crassamura* isolate (GB accession KP863493) or differentiated into a unique haplotype (iib) differentiated from other *P. crassamura* isolates by a single nucleotide polymorphism (SNP) at position 119 (Table 2). *P. megasperma*-complex isolates had 2 SNPs compared to the *P. megasperma* ex-type (GenBank isolate HQ643275), 3 SNPs compared to the *P. crassamura* ex-type, or 1 SNP compared to another *P. megasperma* isolate (GenBank isolate KP863491, Fig. 3).

3.7. Grouping isolates based on COX 1

Aligned COX 1 sequences were each 893 characters long with no indels. A comparison of the North American (NA) *P. crassamura* sequences from California with Italian ex-type and Australian isolate sequences revealed a total of 12 SNPs. All NA haplotypes differed from the *P. crassamura* ex-type haplotype (KP863485) from Italy (Fig. 4). The grouping of these SNPs from isolates in NA, Italy, and Australia revealed a total of 6 haplotypes, 1 being the unique ex-type from Italy, 4 unique to NA, and 2 unique to Australia. The 4 NA haplotypes were i, iia, iib, and iii, and the 2 Australian haplotypes were iic and iid. Compared to the *P. crassamura* ex-type sequence (KP863485), haplotype i differed by 5 SNPs, iia by 8 SNPs, iib by 7 SNPs, and iii by 4 SNPs (Table 2).

3.8. Grouping haplotypes based on oogonial size

The average mean size of oogonia discriminated among the type *P. megasperma*, the i California haplotype of *P. crassamura*, and the iib California haplotype of *P. crassamura* (f-statistic $F_{2,108} = 137.5$, p-value < 0.0001). Size estimates are summarized in Table 3, and a typical oogonium for each examined isolate is shown in Fig. 5. On average, haplotype i had the smallest oogonia and *P. megasperma* had the largest, with haplotype iib being in the middle. The *P. megasperma* isolates used in the morphology test had sequences that matched the *P. megasperma* ex-type ITS and COX 1 sequences.

3.9. Fungicide results

All average EC50 values from the *P. crassamura* were lower than those from *P. megasperma* isolated from planted wildland *D. aurantiacus* (Table 4). The average EC50 value for one of the *P. megasperma* from *D. aurantiacus* was at least an order of magnitude larger than the rest of the isolates. In contrast, the average EC50 value of one of the *P. crassamura* was at least an order of magnitude lower than the rest of the isolates. At the highest fungicide concentration (100 μg/mL) tested, *P. crassamura* haplotype iib had the lowest percent growth. The *P. crassamura* haplotype i isolates from *A. rubra* and *J. effusus* in nurseries, and a *P. megasperma* isolate from *D. aurantiacus* in wildlands had the greatest percent growth. These and the rest of the *P. megasperma* isolates from *D. aurantiacus*, and possibly one of the isolates from *P. racemosa* (upper CI 11 %), had greater than 10 % growth (intermediate sensitivity) and should be considered tolerant.

3.10. Pathogenicity results from stem inoculations on P. racemosa

Isolates obtained from *P. racemosa* all belonged to the California haplotype i of *P. crassamura*. The size of cankers resulting from stem inoculations treatments using haplotype i *P. crassamura* isolates was statistically different from the size of lesions in mock-inoculated controls (f-statistic $F_{3,\ 23} = 6.62$, *p*-value $= 0.002$). The average canker size (mm^2) and SD for the control was 30 ± 6 and for each isolate was: PLRA $= 176 \pm 67$, PLRADsl1a $= 301 \pm 506$, and PLRA-1c $= 71 \pm 14$. PLRADsl1a produced the largest canker ($1540\ mm^2$) and some of the smallest cankers as well ($54\ mm^2$), resulting in a large SD (Fig. 6). Post-inoculation isolations from plants were all successful, and in no case was the pathogen isolated from control treatments. Typical oospores formed on the isolation plates following a 10 d incubation period at 18 °C, and thus, identification could be morphologically confirmed with confidence.

4. Discussion

To the best of our knowledge this is the first official published report of *P. crassamura* from the 14 plant species listed in this paper (Table 1). Although possibly *P. crassamura* isolates may have been included in the study by Yang et al., (2017); likely candidate isolates were identified *as P. megasperma*-like, and no ITS and COX1

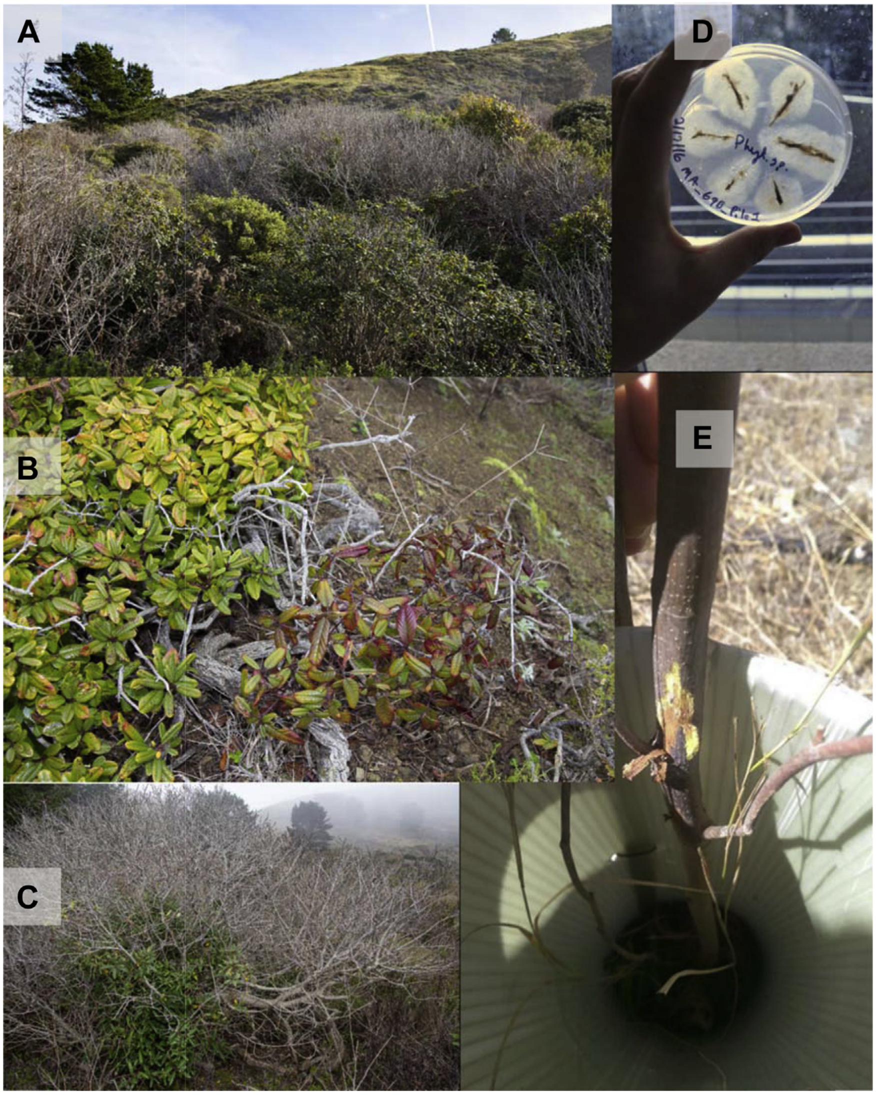

Fig. 1. Disease of *Frangula californica* (A–C) and *Platanus racemosa* (E) associated with *Phytophthora crassamura* in restorations. Colonies of *P. crassamura* (D). Image credit: A,C, William Suckow; B, D, Laura Sims; E San Francisco Public Utilities Commission.

Fig. 2. Stem cankers on *Diplacus aurantiacus* from which *P. megasperma* was isolated.

Table 2
Positions of the single nucleotide polymorphisms (SNPs) observed in the nuclear and mitochondrial sequences of isolates of *P. crassamura* from North America (NA) compared to the ex-type from Italy, and others from Australia.

SNP[a]	Haplotypes						
	Italy	Australia		North America			
	ex-type	iic	iid	i	iia	iib	iii
ITS							
119	G	G	G	G	G	**R**	G
COX-1							
48	C	**T**	C	C	C	C	C
225	G	G	G	G	G	G	**A**
288	T	**C**	**C**	**C**	**C**	**C**	**C**
300	T	**C**	**C**	T	**C**	**C**	**C**
339	A	**C**	**C**	**C**	**C**	**C**	A
441	G	**A**	**A**	G	**A**	**A**	G
480	C	C	C	C	**T**	C	C
633	T	**C**	**C**	**C**	**C**	**C**	T
750	A	A	A	T	A	A	A
813	T	T	T	T	T	T	**A**
825	A	**T**	**T**	**T**	**T**	**T**	A
864	C	**T**	**T**	**T**	**T**	**T**	C

[a] Single nucleotide polymorphisms (SNPs) at the indicated base position compared to the ex-type isolate. SNPs are boldfaced where they differ from the ex-type.

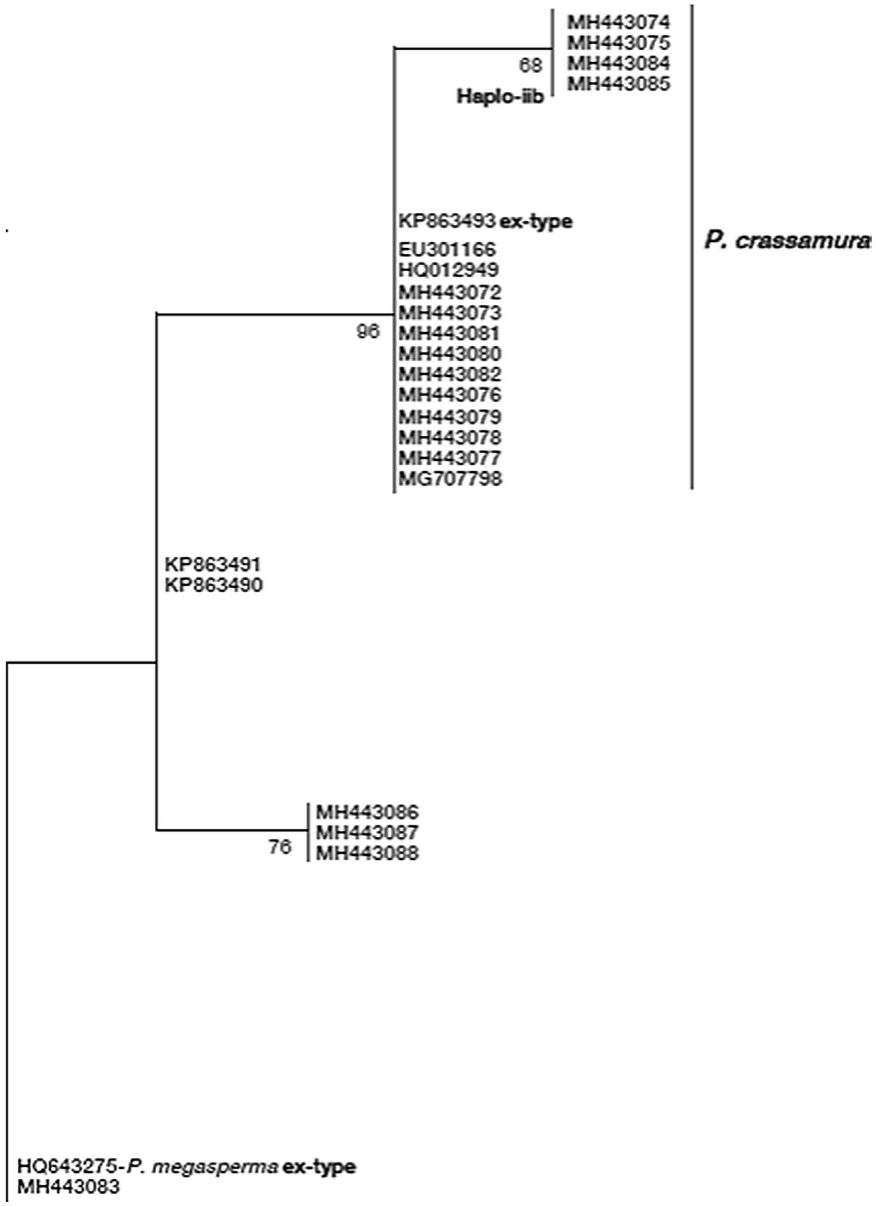

Fig. 3. Internal transcribed spacer (ITS) phylogeny displaying relationships between *P. crassamura* and *P. megasperma* isolates. Branch support is displayed below each branch.

sequences were reported to serve as a term of comparison. *P. crassamura* was always more abundant (range 1.5–10 times, depending on County) than all of the other *P. megasperma* -complex isolates combined, and was found in a total of 13 restoration sites in 6 counties in the San Francisco Bay Area (California). Only *P. crassamura* was found in Monterey and Alameda Counties, although the absence of other *P. megasperma* species complex taxa in these two counties may have been due to the lower sampling effort. *P. megasperma* complex isolates (including *P. crassamura*) were overall most frequently collected from wildlands restored with planted stock, and from stockpiled trail soil that contained untreated 'recycled' contractor plant waste. This result suggests that *P. crassamura* and *P. megasperma* found in restored sites originated from infected plants and contaminated plant containers coming from infested nurseries. This is also supported by the fact that *P. crassamura* was not reported from wildlands in the USA until recently, and that isolates belonging to both taxonomic groups are only now being reported in several California plant production facilities that provide plant stock for restoration projects here and in Sims et al. (2018).

One of the aims of this study was to differentiate taxa within the *P. megasperma* species complex found during surveys around the San Francisco Bay Area based on DNA sequences, oogonia size, morphology, and sensitivity to mefenoxam. A further aim was to determine whether taxa from plant production facilities were identical to those in restored wildlands, in order to provide further evidence in support of a facility source of isolates obtained from restorations. Our analyses unexpectedly revealed that, while the majority of isolates could be putatively defined as *P. crassamura* based on gross morphology and phylogenetic placement, they belonged to two ITS and four COX 1 haplotypes. California isolates could be assigned to one of four lineages, molecularly different from the *P. crassamura* described from Italy by Scanu et al (2015). One group (group iib) had a unique sequence at both loci, while the other three groups (i, iia, iii) shared an identical ITS sequence with *P. crassamura* isolates from Italy but had a different COX 1 sequence.

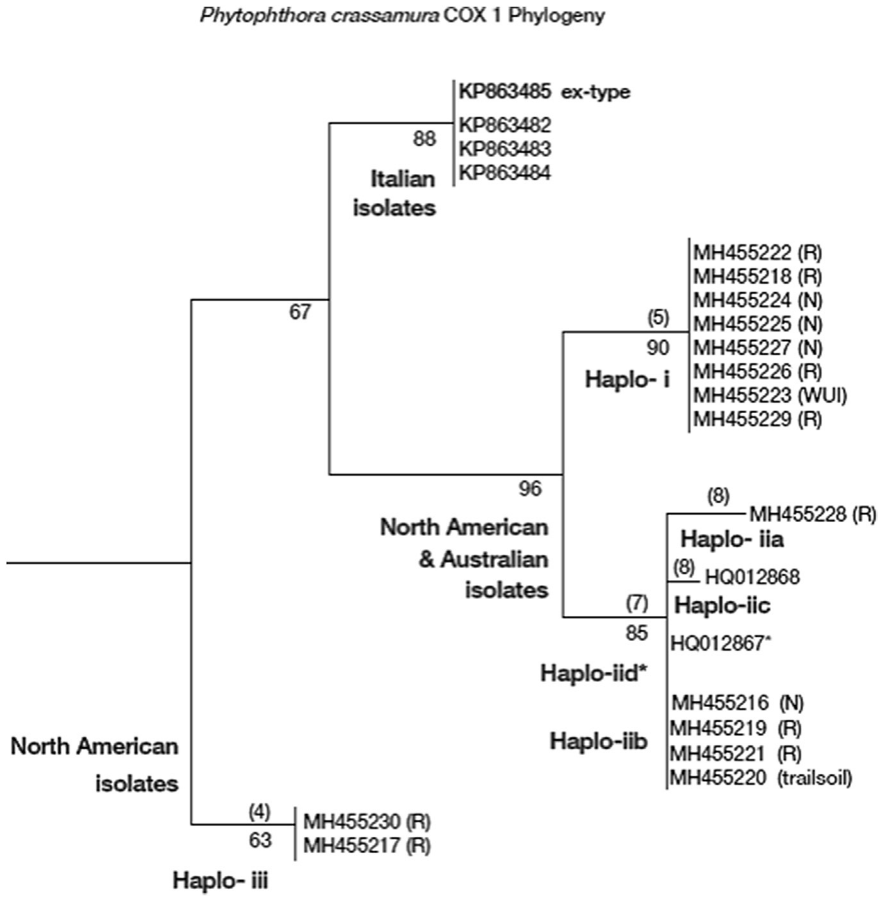

Fig. 4. *Phytophthora crassamura* COX1 phylogeny displaying North American (NA) haplotypes and references. Haplo-i, iia, iib, and iii are for the respective haplotypes found in NA. Isolates from Italy, NA, and Australia are included and labelled on the subtending branch. Branch support is displayed below each branch, and number of bp differences compared to the ex-type sequence are displayed in parenthesis above the branch. *Phytophthora megasperma* ex-type sequence was used as the outgroup.

Three groups appeared to be sister taxa to *P. crassamura* from Italy, while one was potentially ancestral. Mitochondrial regions are not subject to recombination and thus are ideal to reconstruct the evolutionary histories of living organisms, as exemplified by studies on humans (Kogelnik et al., 1996). Notwithstanding that more loci should be sequenced to define in more detail the evolutionary relationships among the four California lineages and *P. crassamura* from Italy, the data presented here suggests not only that *P. crassamura* may be more widespread than originally thought, but also that it may be diversifying into distinct taxonomic entities and possibly novel species. The similarity or identity of ITS sequences suggests these taxa representing *P. crassamura* lineages may either have evolved recently or may not have attained reproductive isolation yet and may still have the ability to mate and exchange nuclear genomes. Mating is likely to occur in plant nurseries or plant production facilities where often multiple *Phytophthora* species may be present on plants with an extremely varied geographic origin. We refer here to the four lineages closely related to *P. crassamura* from Italy which are described in this paper. The fact that oogonia sizes were distinctive among two of the *P. crassamura* lineages and separate from *P. megasperma* sensu stricto supports them as unique lineages among *P. crassamura*. Other morphological characteristics such as temperature ranges for mycelial growth, and space between oospore and oogonium have been shown to have diagnostic value within the *P. megasperma* complex (Scanu et al., 2015) and could have allowed for a better differentiation of

Table 3
Comparisons of oogonia of *P. crassamura* haplotypes i and iib with those of *P. megasperma* including the estimated average mean diameters, median oogonia diameters, the range of oogonia sizes observed, and the estimated average mean diameter for each isolates based on where they were sampled (Nursery (N), Restoration (R), trail stockpile, or the wildland–urban interface (WUI)). Average mean estimates include 95 % confidence intervals.

COX 1 group	*P. crassamura*		*P. megasperma*
	haplotype i	halpotype iib	type
Breeding system	homothallic	homothallic	homothallic
Estimated average mean diameters (μm)	36.2 ± 0.9	41.9 ± 0.9	48.2 ± 1.1
Median diameters	37	41	49
Diameter ranges	30–44	38–49	40–53
Diameters per location Restoration (R)/Nursery (N)/Other (specified)	N 37.3 ± 1.4; WUI 35.2 ± 1.4	N 42.6 ± 1.3; R 41.2 ± 1.3	WUI 48.4 ± 1.8[a]; trail stockpile 48.2 ± 1.3
Antheridial attachment	only paragynous	mostly paragynous	mostly paragynous

[a] Some possibly aborted.

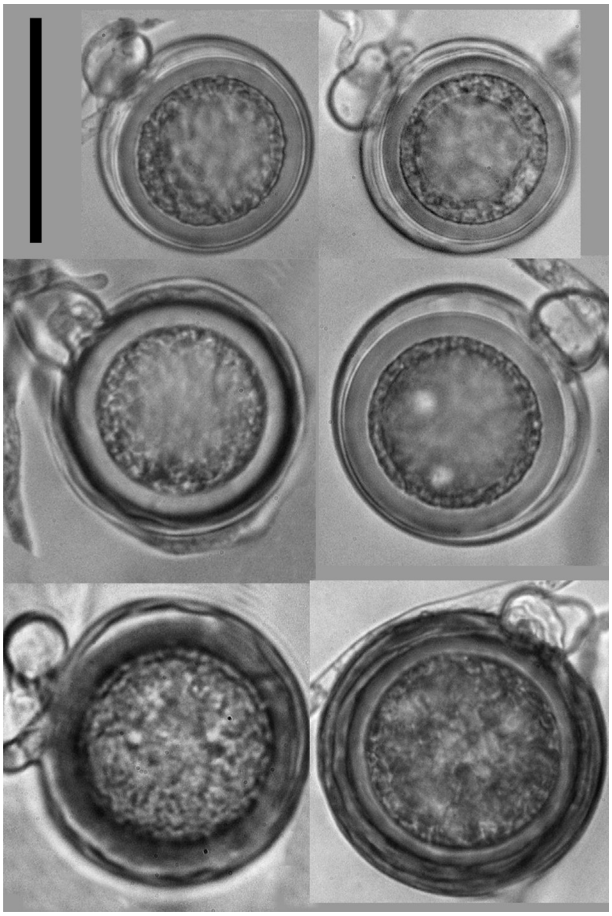

Fig. 5. Typical oogonia and spores of *Phytophthora crassamura* i (top row), iib (middle row), and *Phytophthora megasperma* (bottom row). One from each isolate examined is shown. Bar = 40 μM.

lineages, however oogonia size was an excellent diagnostic morphological character, and has been used previously to differentiate biologically relevant taxa within the *P. megasperma*-complex (Sansome and Brasier, 1974; Waterhouse, 1963). To our knowledge, this is one of the first studies to use DNA sequence-based haplotype information combined with oogonia size to evaluate taxonomically relevant groups within *P. crassamura*, a combined approach whose importance has been in part discussed by Scanu et al. (2015) and by Yang et al., (2017).

A further aim of this study was to provide evidence corroborating that isolates from restoration sites are the same as those found in plant production nurseries, thus validating that the infested nurseries may be an important source of these pathogens introduced in the wild. *P. crassamura* lineages belonging to groups i and iib were found both in nurseries and in restorations, and isolates from each of the two lineages were all characterized by the same DNA sequences at both the ITS and COX 1 loci, by the same oogonia size, and by the ability to grow even in the presence of high concentrations of mefenoxam. Although it could be argued that multi locus genotyping may be needed to irrefutably make this connection (Croucher et al., 2013), both phenotypic and genotypic evidence presented here strongly suggest *P. crassamura* isolates in restoration sites are the same as those found in plant production facilities which provide the plant stock to be used for restorations. The fact that *P. megasperma* complex isolates (for instance *P. crassamura* isolates from haplotype iib) displayed a great degree of growth difference in the presence of mefenoxam, even among isolates from a single host and within a single restoration site, strongly suggests a history of exposure to the chemical. Cumulatively, these results corroborate a nursery origin not just for *P. crassamura* but for *P. megasperma* sensu lato isolates found in restorations.

The final aim of this study was to investigate whether "*P. megasperma*-like" isolates including *P. crassamura* lineages may be characterized as being virulent or not. Our study provides several lines of evidence that *P. crassamura* lineages and *P. megasperma* may become serious pathogens, at least on some hosts, but also highlights an intrinsic difficulty in proving a direct and "exclusive" effect of this taxon on native California flora. *P. crassamura* lineages were collected in 20 % of sites in Santa Clara County and, although it was not found to be linked to any particular disease, it was found among restoration plantings in decline (Bourret et al., 2018). Even if one *P. crassamura* lineage (iii) stood out as the second most commonly isolated *Phytophthora* species after *P. cactorum* at these sites, the reason why it may have often been difficult to link *P. crassamura* with disease in these settings could be due to the large number of hosts and other *Phytophthora* species present in these sites, making a direct correlation difficult to establish. In Marin County, *P. megasperma* not *P. crassamura* was primarily isolated from the stems and roots of *D. aurantiacus* with mortality evident in "root disease mortality centers" (Hansen and Goheen, 2000) characterized by circles of dead and dying plants with diameters as large as 10 m, suggesting that a few plants may have been infected initially and then disease spread outward from these over time. In San Mateo County, *P. crassamura* isolates were obtained from diseased *F. californica*. However, the observed disease symptoms may have been

Table 4
Fungicide test results for isolates of the two *P. crassamura* haplotypes and isolates *of* P. megasperma found in nurseries (**N**) restorations (**R**) and from stock-piled soil (**TS**). Letters represent statistical groupings.

Phyt. species	Host	Location	% Growth[a]; statistical group	EC50	Models used for EC50
P. crassamura i	*Platanus racemosa*	R	7 ± 1; a	0.04 ± 0.02; b	Log normal (1), Log logistic (2), Weibull (2)
P. crassamura i	*P. racemosa*	R	9 ± 2; ab	0.03 ± 0.02; b	Log logistic (2), Weibull (2)
P. crassamura i	*Alnus rubra*	N	18 ± 1; c	0.02 ± 0.01; b	Weibull (1)
P. crassamura i	*Juncus effusus*	N	18 ± 1; c	0.02 ± 0.015; b	Weibull (1)
P. crassamura iib	*Frangula californica*	R	6 ± 1; a	0.0011 ± 0.0009; a	Weibull (1)
P. crassamura iib	*F. californica*	R	4 ± 2; a	0.02 ± 0.01; b	Log normal (1), Log logistic (2), Weibull (2)
P. crassamura iib	*Pinus torreyana*	N	5 ± 1; a	0.01 ± 0.005; b	Log normal (1), Log logistic (2), Weibull (2)
P. crassamura iib	na	TS	6 ± 2; a	0.02 ± 0.01; b	Log normal (1), Log logistic (2), Weibull (2)
P. megasperma	*Diplacus aurantiacus*	R	6 ± 1; a	0.07 ± 0.01; c	Log normal (1), Log logistic (2), Weibull (2)
P. megasperma	*D. aurantiacus*	R	13 ± 2; b	0.08 ± 0.01; c	Log normal (1), Log logistic (2), Weibull (2)
P. megasperma	*D. aurantiacus*	R	10 ± 2; b	0.07 ± 0.01; c	Log normal (1), Log logistic (2), Weibull (2)
P. megasperma	*D. aurantiacus*	R	22 ± 2; d	0.74 ± 0.13; d	Log normal (1), Log logistic (2), Weibull (2)

[a] % Growth was the amount compared to the control and fungicide concentration 100 μg/mL.

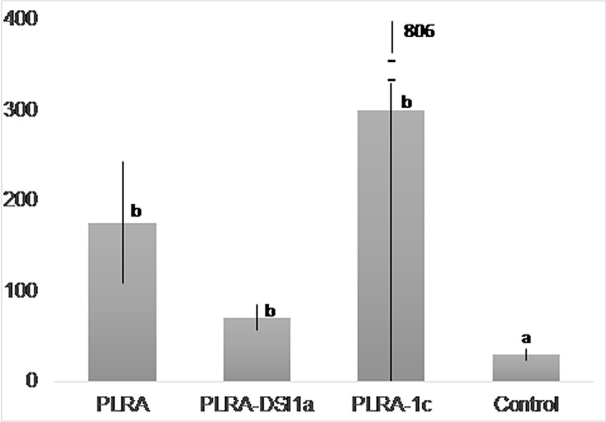

Fig. 6. Canker area (mm^2) caused by each of three isolates of *Phytophthora crassamura* haplotype i on *Platanus racemosa* in a greenhouse trial.

caused by the co-presence of other pathogens simultaneously isolated from the same plants. *Phytophthora multivora* and *Fusarium lateritium* were in fact isolated together in one area, and *P. crassamura and F. lateritium* in another: further studies need to determine which ones of the three microbes may be primary pathogens, and which may be secondary ones facilitated by the poor health conditions caused by the primary pathogen(s). In contrast to what was observed in Santa Clara County, San Mateo, and Marin Counties, severe disease symptoms were associated with the exclusive isolation of *P. crassamura* lineage i from *P. racemosa* (California sycamore) in Alameda County. *P. crassamura* haplotype i appeared to cause stem cankers on California sycamore resulting in severe above-ground dieback. The height at which cankers were observed on stems varied, possibly depending on the extent of splash of infested soil. Symptoms on roots were also present and consisted of typical water-soaked lesions, but were rather limited in severity compared to the observed stem cankers. Our inoculation experiment not only completed Koch's postulate for the *P. racemosa* – *P. crassamura* lineage i host–pathogen combination but clearly indicated *P. crassamura* could be a primary pathogen on young *P. racemosa* trees.

In conclusion, this study shows that COX 1 DNA sequence and oogonia size can be used to identify unknown and yet undescribed taxa within the *P. megasperma* species complex: these may be closely-related to the recently described *P. crassamura* but are phylogenetically (and also morphologically for two of four novel groups) distinct lineages. We show that at least four distinct novel *P. crassamura* lineages are prevalent in restoration sites across the San Francisco Bay Area, and we provide multiple lines of evidence indicating that such isolates were introduced through the use of infected plant stock grown in infested nurseries. The presence of significant disease and our ability to re-create disease on California sycamore by inoculating healthy seedlings with *P. crassamura* lineage i isolates and its presence in relation to disease of several other hosts, indicates that *P. crassamura* lineage i represents a threat to native California plants. Due to the findings presented here, we conclude that the further spread of any *P. crassamura* lineage should be limited through monitoring efforts, the use of mitigation strategies, and by employing pathogen-free plant stock for restoration efforts (Frankel et al., 2016; Sims et al., 2016).

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References

Baum, D.A., Smith, S.D., 2013. Tree Thinking: an Introduction to Phylogenetic Biology. Macmillan Publishing, p. 175.

Bourret, T.B., Mehl, H.K., Swiecki, T.J., Bernhardt, E.A., Hillman, J.M., Rizzo, D.M., 2018. Restoration Outplantings of Nursery-origin Californian Flora Are Heavily Infested with *Phytophthora*. Dissertation chapter, University of California, Davis.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G., Brasier, C.M., 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet. Biol. 30, 17–32.

Croucher, P.J., Mascheretti, S., Garbelotto, M., 2013. Combining field epidemiological information and genetic data to comprehensively reconstruct the invasion history and the microevolution of the sudden oak death agent *Phytophthora ramorum* (Stramenopila: oomycetes) in California. Biol. Invasions 15, 2281–2297.

Drechsler, C., 1931. A crown-rot of hollyhocks caused by *Phytophthora megasperma* n. sp. J. Wash. Acad. Sci. 21, 513–526.

Erwin, D.C., 1954. Root rot of Alfafa caused by *Phytophthora cryptogea*. Phytopathology 44, 700–704.

Erwin, D.C., 1965. Reclassification of the causal agent of root rot of alfalfa from *Phytophthora cryptogea* to *Phytophthora megasperma*. Phytopathology 55, 1139–1143.

Faris, M.A., Sabo, F.E., Barr, D.J.S., Lin, C.S., 1989. The systematics of *Phytophthora sojae* and *P. megasperma*. Can. J. Bot. 67, 1442–1447.

Frankel, S.J., Alexander, J., Appel, J., Benner, D., Bernhardt, E., Blomquist, C., Bourret, T., Garbelotto, M., Hillman, J.M., Ingolia, M., Kosta, K., Lyman, G., Mehl, H., Natesan, E., Rizzo, D.M., Rooney-Latham, S., Shor, A., Sims, L.L., Suslow, K., Swiecki, T., 2016. Assembling a response to inadvertent *Phytophthora* plant pathogen introductions in restoration areas: the working group on *Phytophthoras* in native plant habitats. In: SERCAL: the 23rd Annual Conference of the California Society for Ecological Restoration; May 12; Lake Tahoe.

Garbelotto, M., Frankel, S.J., Scanu, B., 2018a. A Review of the Genus *Phytophthora* with an Emphasis on Soilborne and Waterborne Species, Including a List of Species Recently Isolated from Some Northern California Restoration Sites and Restoration Nurseries. California Agriculture (in press).

Garbelotto, M., Sims, L.L., Swiecki, T., Quinn, M., Bernhardt, E., Oliver, L., Popenuck, T., 2018b. Three Cost-effective and Reliable Approaches for the Detection of *Phytophthora* Species in Plant Production Facilities. California Agriculture (in press).

Gelman, A., Rubin, D., 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7, 57–511.

Gibbs, J.N., Lipscombe, M.A., Peace, A.J., 1999. The impact of Phytophthora disease on riparian populations of common alder (*Alnus glutinosa*) in southern Britain. Eur. J. For. Pathol. 29, 39–50.

Gisi, U., Cohen, Y., 1996. Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. Annu. Rev. Phytopathol. 34, 549–572.

Gisi, U., Sierotzki, H., 2008. Fungicide modes of action and resistance in downy mildews. Eur. J. Plant Pathol. 122, 157–167.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows. Nucleic Acids Symp. Ser. 41, 95–98.

Hansen, E.M., Goheen, E.M., 2000. *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in western North America. Annu. Rev. Phytopathol. 38, 515–539.

Hansen, E.M., Wilcox, W.F., Reeser, P.W., Sutton, W., 2009. *Phytophthora rosacearum* and *P. sansomeana*, new species segregated from the *Phytophthora megasperma* "complex. Mycologia 101, 129–135.

Hildebrand, A.A., 1959. A root and stalk rot of soybeans caused by *Phytophthora megasperma* Drechsler var. *sojae* var. nov. Can. J. Bot. 37, 927–957.

Hu, J., Hong, C., Stromberg, E.L., Moorman, G.W., 2010. Mefenoxam sensitivity in *Phytophthora cinnamomi* isolates. Plant Dis. 94, 39–44.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Inderjit, Streibig J.C., Olofsdotter, M., 2002. Joint action of phenolic acid mixtures and its significance in allelopathy research. Physiol. Plantarum 114, 422–428.

Jeffers, S.N., Aldwinkle, H.S., 1987. Enhancing detection of *Phytophthora cactorum* in naturally infested soil. Phytopathology 77, 1475–1482.

Jung, T., Jung, M.H., Cacciola, S.O., Cech, T., Bakonyi, J., Seress, D., Mosca, S., Schena, L., Seddaiu, S., Pane, A., Magnano di San Lio, G., Maia, C., Cravador, A., Franceschini, A., Scanu, B., 2017. Multiple new cryptic pathogenic *Phytophthora* species from *Fagaceae* forests in Austria, Italy, and Portugal. IMA Fungus 8, 219–244.

Kang, S., Kodell, R.L., Chen, J.J., 2000. Incorporating model uncertainties along with data uncertainties in microbial risk assessment. Regul. Toxicol. Pharmacol. 32, 68–72.

Kogelnik, A.M., Lott, M.T., Brown, M.D., Navathe, S.B., Wallace, D.C., 1996. MITOMAP: a human mitochondrial genome database. Nucleic Acids Res. 24, 177–179.

Martin, F.N., 2000. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. Mycologia 92, 711–727.

Martin, F.N., Tooley, P.W., 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95, 269–284.

McIntosh, D.L., 1964. *Phytophthora* spp. in soils of the Okanagan and Similkameen valleys of British Columbia. Can. J. Bot. 42, 1411–1415.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. Nlme: linear and nonlinear mixed effects models. R Packag. Vers. 3, 1–137.

R Core Team, 2018. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M.Z., Uematsu, S., Coffey, M.D., Uzuhashi, S., Suga, H., Kageyama, K., 2014. Re-evaluation of Japanese *Phytophthora* isolates based on molecular phylogenetic analyses. Mycoscience 55, 314–327.

Rahman, M.Z., Uematsu, S., Kimishima, E., et al., 2015. Two plant pathogenic species of *Phytophthora* associated with stem blight of Easter lily and crown rot of lettuce in Japan. Mycoscience 56, 419–433.

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, e0146021.

Ronquist, F., Huelsenbeck, J.P., van der Mark, P., 2011. MrBayes 3.2 Manual. http://mrbayes.sourceforge.net/mb3.2_manual.pdf. (Accessed April 2016).

Rooney-Latham, S., Blomquist, C.L., 2014. First report of root and stem rot caused by *Phytophthora tentaculata* on *Mimulus aurantiacus* in North America. Plant Dis. 93, 843.

Rooney-Latham, S., Blomquist, C.L., Swiecki, T., Bernhardt, E., Frankel, S.J., 2015. First detection in the US: new plant pathogen, *Phytophthora tentaculata*, in native plant nurseries and restoration sites in California. Native Plants J. 16, 23–27.

Sansome, E., Brasier, C.M., 1974. Polyploidy associated with varietal differentiation in the megasperma complex of *Phytophthora*. Trans. Br. Mycol. Soc. 63, 461–467.

Scanu, B., Linaldeddu, B.T., Deidda, A., Jung, T., 2015. Diversity of *Phytophthora* species from declining Mediterranean maquis vegetation, including two new species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. PLoS One 10, e0143234.

Sims, L.L., Sutton, W., Reeser, P., Hansen, E.M., 2015. The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. Mycologia 107, 889–902.

Sims, L.L., Conforti, C., Gordon, T., Larssen, N., Steinharter, M., 2016. Presidio *Phytophthora* Management Recommendations. https://nature.berkeley.edu/garbelottowp/?page_id=3303. (Accessed May 2017).

Sims, L.L., Tjosvold, S., Chambers, D., Garbelotto, M., 2018. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathol. https://doi.org/10.1111/ppa.12933.

Tompkins, C.M., Tucker, C.M., Gardner, M.W., 1936. Phytophthora root rot of cauliflower. J. Agric. Res. 53, 685–692.

Tyler, B.M., 2006. *Phytophthora sojae*: root rot pathogen of soybean and model oomycete. Mol. Plant Pathol. 8, 1–8.

Waterhouse, G.M., 1963. Key to the Species of *Phytophth*ora de Bary. Mycological Papers, vol. 92. Commonwealth Mycological Institute, Kew, UK.

Weisberg, S., 2005. Applied Linear Regression, third ed. John Wiley & Sons, New York.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications, pp. 315–322. London.

Wortley, L., Hero, J.M., Howes, M., 2013. Evaluating ecological restoration success: a review of the literature. Restor. Ecol. 21, 537–543.

Yang, X., Tyler, B.M., Hong, C., 2017. An expanded phylogeny for the genus *Phytophthora*. IMA Fungus 8, ,355–384.