HIERARCHICAL ANALYSIS AND DIVERSITY STUDIES OF *XYLELLA FASTIDIOSA* POPULATIONS IN CALIFORNIA BY MULTI-LOCUS SIMPLE SEQUENCE REPEAT MARKERS

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ABSTRACT
Xylella fastidiosa (*Xf*) is the causative agent of Pierce's disease (PD) in grapevine. Using 18 simple sequence repeat (SSR) markers, we assessed variation within and between populations of *Xf* isolated from grapevine in California. Eighty-three *Xf* isolates from 15 populations present in four regions of California were evaluated for sequence variation. Average genetic diversity was substantial (H = 0.807, I=0.5385) across the 18 loci examined. Diversity within populations varied from 0.227±0.058 (population *Sangiacom*) to 0.583±0.63 (population *Temecula*) but was not dependent upon the grape cultivar serving as host. Analysis of Molecular Variance (AMOVA) indicated no hierarchical population structure, as 98% of the variation was attributed to within population diversity. Higher order variation was minor; diversity among populations or among regions each accounted for only 1% of the variation observed. Principal component analysis (PCA) indicated that only ~53% of the variation was explained by the first three components, further suggesting a lack of hierarchical population structure in *Xf* infecting grape in California. Collectively, these results indicate high levels of gene flow between populations and further suggest that strong selection may dominate other population genetic forces responsible for structuring *Xf* populations resident in Californian grapes. We also initiated similar studies for almond specific *Xf* strains to better understand host species specific diversity patterns and population structure, if any, for almond *Xf* strains. While acquiring and preparing infected almond samples for further SSR analysis, we observed that seasonal variation plays an important role in extent of *Xf* infection.

INTRODUCTION
California has one of the most productive agricultural ecosystems in the United States. Yet, limited genetic variation among crop cultivars and monoculture practices may impose directional selection on pathogen populations. Host plant resistance is a critical component of integrated crop management. Changes in pathogen population structure or virulence can lead to host resistance breakdown. Therefore, understanding pathogen genetic diversity is critical for development of effective disease control strategies. An important aspect of plant pathogen population genetics is the extent to which populations are subdivided, either geographically or by host species/cultivar. The goal of this project is to analyze *Xylella fastidiosa* (*Xf*) haplotypes generated by SSR genotyping within a hierarchically structured (individual plants, individual vineyards, and distinct growing regions) sampling strategy to understand pathogen population dynamics in grapevine hosts at various levels. Previously, we reported development of multilocus simple sequence repeat (SSR) markers for *Xf* population genetic analysis. This marker system appears to be sensitive in detection and powerful in discriminating *Xf* genotypes. This marker system also provides high throughput capability for a large scale sampling and analyses.

OBJECTIVES
1. Analyze *Xf* strain variation and diversity specific to individual host grapes, including hierarchical analysis of population subdivision to understand population differentiation in host-specific pathogen populations.
2. Study the effect of host cultivar on structure of pathogen populations.
3. Evaluate pathogen population dynamics in almond-specific *Xf* strains from three almond populations.

RESULTS AND DISCUSSION

Objective 1.

We analyzed genetic diversity and geographic population structure of *Xf* in Californian vineyards (Napa, Sonoma, Kern and Riverside counties). Results based on multi-locus SSR marker systems and hierarchical sampling showed that all 18 SSR primers were able to discriminate among *Xf* strains (N=83), revealing diversity with a mean H value of 0.609 across all loci. Diversity ranged from a low (H=0.093) for the loci GSSR6 and ASSR 19 to a high of H= 0.818 for locus OSSR 11. The average genotypic richness (no. of polymorphic loci) was high (67%) across populations. However variation was not as evenly distributed, ranging from a low of 38.9% for population Clos du Bois to a high of 88.9% for populations Monticello and Temecula.

The hierarchical datasets allowed partitioning of genetic differentiation among regions, within a region among populations, and within population among individuals. Analysis of Molecular Variance (AMOVA) results revealed 1% diversity among different regions and 1% among populations within a region. Nearly all of the diversity (98%) was represented by individual strains within populations (Table 1). Lack of clear genetic structure was also evidenced by Principal Component Analysis (PCA), where 53.13% of variation was explained by first three components across the hierarchical structure. The conclusion from the current 18 SSR loci analysis doesn't agree with the analysis based on 6 SSR loci reported earlier (Lin et al., 2005). The discrepancy may be due to the fact that when only a few loci were used, each locus contributes significant weight to the overall genetic variation, which could overestimate genetic variation. Absence of genetic differentiation observed among grape *Xf* populations in the present study is supported by the observations of Schuenzel et al (2005), in which no genetic differentiation among northern and southern California populations of *Xf* from grapes was observed. We hypothesize that the reasons for this may include relatively recent spread of the pathogen and the geographic range of insects vectoring the pathogen. The values for overall gene flow (Nm =2.2272) supported lack of population differentiation. Sonoma region showed least gene flow (Table 2) compared to highest for Temecula region.

Objective 2

In this study, observed genetic variation of *Xf* strains was not related to or dependent on the different grape cultivars from which the strains were collected. Preliminary cultivar effect results indicated that the type of grape cultivar did not play any role in grouping *Xf* populations from various counties. We observed that *Xf* isolated from Chardonnay grown in Sonoma county grouped with other *Xf* strains isolated from other grape varieties grown within Sonoma county rather than grouping with *Xf* isolated from Chardonnay grown in Napa or Temecula counties (Figure 1). Although this is the first report to this effect in grape, similar results were observed for *Xf* isolated from citrus; e.g., different sweet orange varieties did not affect population structure of *Xf* in Brazil (Coletta-Filho and Machado (2002). However, further research of this effect in grape is necessary before concluding that grape cultivar does not affect *Xf* population structure.

Objective 3

Using the same SSR marker system, we extended our investigation to almond leaf scorch (ALS) disease in California's San Joaquin Valley. The seasonal collection and detection studies (Figure 2) showed that *Xf* populations were low in early season (March and April), when *Xf* is less easily detected with PCR. *Xf* populations quickly increased with increased vector activity in late spring/early summer. Successful *Xf* isolation/culture and PCR detection were comparable after July through October. To increase the power of our analysis, we are analyzing the results from 13 additional SSR markers this year (in addition to the five SSR markers used in previous years) from 73 *Xf* strains of almond. Allelic types and allelic frequencies of haplotypes among and within populations are being analyzed to better understand the genetic structure and population dynamics of *Xf* strains in almond.

CONCLUSIONS

Hierarchical sampling and multilocus genetic marker analysis provided informative details of genetic diversity, population structure, and the evolutionary process of selection/adaptation of grape *Xf* strains in grapevine growing regions. Our preliminary conclusions about the lack of a grape cultivar effect on *Xf* population dynamics may be an important addition to the current knowledge of this pathogen in grape. Information from population differentiation within grape specific *Xf* strains facilitates better understanding PD epidemiology and the development of an effective disease management strategy.

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Table 1. Summary of AMOVA analysis with population differentiation parameters.

Source	df	SS	MS	Est. Var.	%	Stat	Value	Prob
Among Regions	8	17.898	2.237	0.013	1%	PhiRT	0.006	0.001
Among Pops/Regions	6	12.909	2.151	0.012	1%	PhiPR	0.006	0.002
Within Pops	68	141.227	2.077	2.077	98%	PhiPT	0.012	0.001
Total	**82**	**172.033**	**6.466**	**2.102**				

Table 2. Population structure parameters of *Xf* strains from four regions of California**.**

Region name	Diversity (H)	Effective # alleles (A_E)	Migration index (Nm)	Private alleles	# of significant LD (p=0.05)
Sonoma	0.4414 ± 0.23	2.08 ± 0.82	0.6050	0.06-0.33 Ave=0.18	0 - 223 Ave=71.3
Napa	0.4005 ± 0.22	1.95 ± 0.88	1.3891	0.06-0.50 Ave=0.39	24-74 Ave=41.2
Kern	0.4789 ± 0.29	2.50 ± 1.30	3.5660	0.22-0.28 Ave=0.25	89-129 Ave= 109
Temecula	0.5422 ± 0.22	2.55 ± 0.88	2000	0.6	180
Total	**0.5347 ± 0.24**	**2.62 ± 1.09**	**2.2271**	**0.36**	100.3

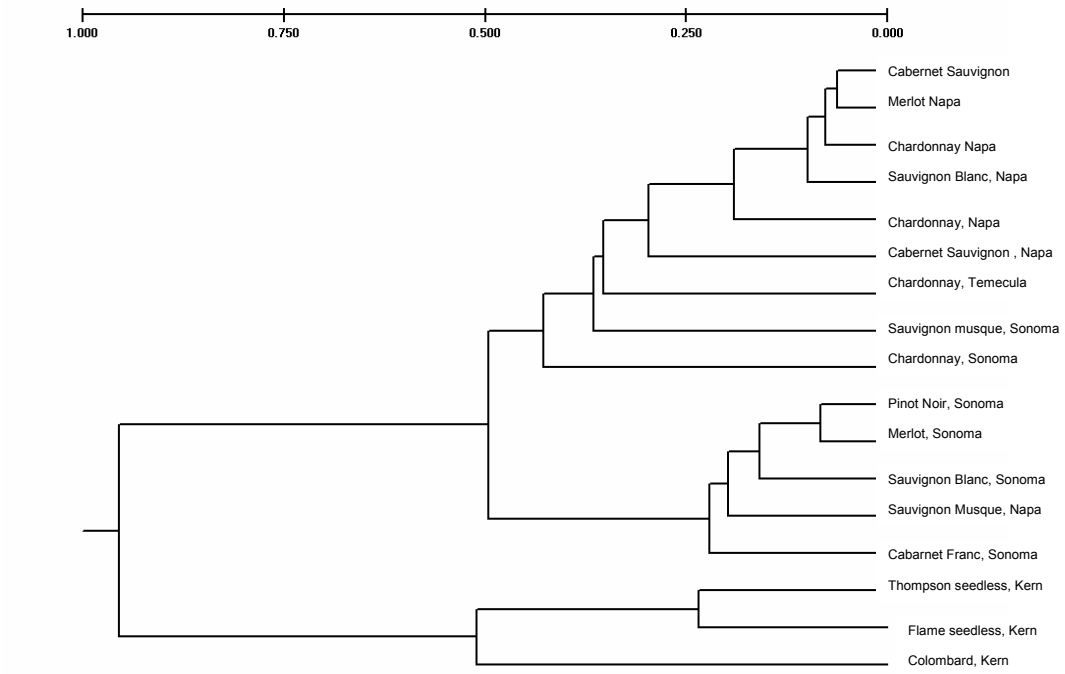

Figure 1. Dendrogram representing grouping of *Xf* strains from four regions according to grape cultivar.

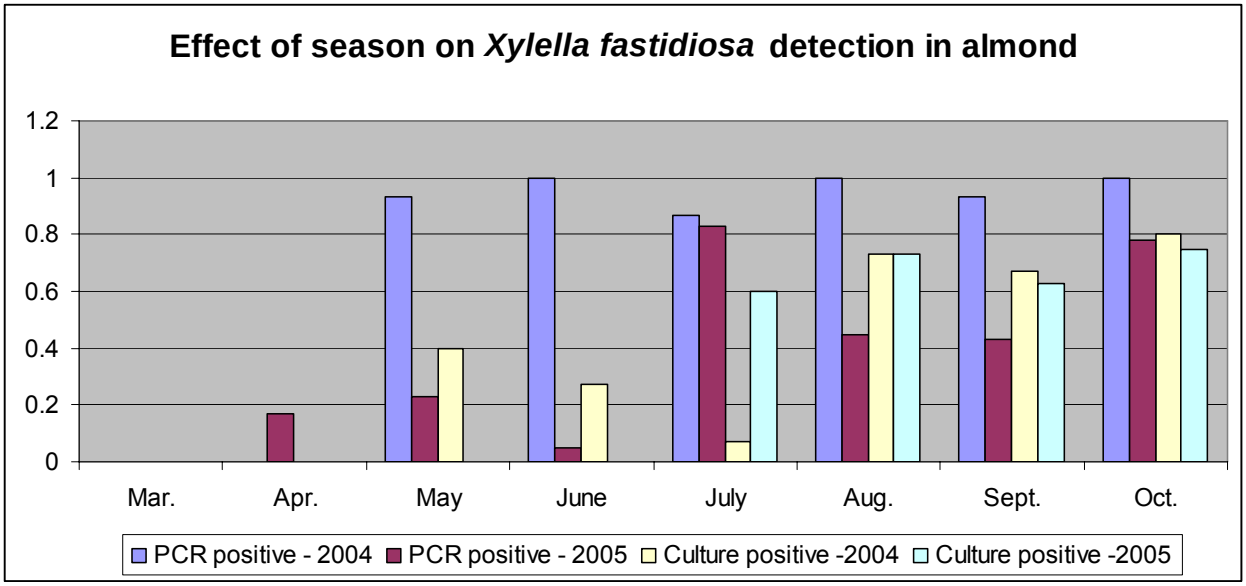

Figure 2. Effect of seasonal variation on PCR and culture based detection levels of *Xylella* infection.