MULTI-LOCUS SEQUENCE TYPING (MLST) TO IDENTIFY RESERVOIRS OF *XYLELLA FASTIDIOSA* DIVERSITY IN NATURAL HOSTS IN CALIFORNIA

Project Leaders:
Robert F. Luck, Richard Stouthamer, and Danel Vickerman
Department of Entomology
University of California
Riverside, CA 92521

Leonard Nunney
Department of Biology
University of California
Riverside, CA 92521

Donald A. Cooksey
Department of Plant Pathology
University of California
Riverside, CA 92521

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ABSTRACT
The ability to identify accurately and track the strains of an important infectious agent causing a plant disease is fundamental to the surveillance and management of that disease. Therefore, it is critical that we determine what type of *Xylella fastidiosa* (*Xf)* disease reservoir exists in uncultivated habitats and identify potential new variants of *Xf* and their host plants. Our plant collection and xylem extraction efforts have targeted native and naturalized plants in riparian habitats that may harbor *Xf* which is spread from cultivated plants such as grapes by infected blue green sharpshooter (BGSS), *Graphocephala atropunctata* (Signoret) as they move from the riparian vegetation into the vineyards and adjacent plant communities (Purcell 1975). In order to detect and characterize the *Xf* strains from field collected plants, we have first developed a protocol using the real-time polymerase chain reaction (PCR), which has been proven to detect *Xf* at much lower levels than is possible with traditional PCR, and is a one step process reducing the possibility of error (Oliveira et al. 2002, Bextine and Miller 2005). Furthermore, we are using a technique for whole genome amplification which allows us to boost the amount of DNA in low concentration samples so that traditional PCR can be used to amplify and sequence a series of genes. We have detected *Xf* and determined the strain in several common native plant species, including *Encelia farinosa* (brittlebush), *Xanthium strumarium* (cocklebur), *Salvia mellifera* (black sage), *Pluchea odorata* (sweetscent) and *Vitis girdiana* (wild grape). This information is essential for fully understanding the potential for recombination and the generation of new strains. The results of this project will allow for a rational control of the pathogen by either planting or removing particular host plants in the proximity of the *Xf* reservoirs.

INTRODUCTION
Little is known about the potential reservoir of *Xf* contained in native and naturalized plants. Since the insect vectors of *Xf* move freely between cultivated and uncultivated areas, an accurate identification of these *Xf* variants is critical to our understanding of the epidemiology of this disease, and hence to its control. We are using the Multilocus Sequence Typing (MLST) as the basis 1) to unambiguously and uniquely characterize *Xf*' strains and clonal diversity, 2) to associate an *Xf* strain and clone with its host plant(s), and 3) to associate an *Xf* strain with its geographic location. MLST is a technique that is currently used to characterize strains and clones of 12 different human bacterial pathogens and to trace their epidemiology based on differences in their nucleotide sequences in one or more genes (http://www.mlst.net/mixc/further.asp) (Maiden *et al*. 1998, Enright and Spratt, 1999). In a related project funded by UC DANR, this technique is being used to document within and among-strain variation in *Xf* using the DNA sequence of seven target genes found in all *Xf* strains (Scally et al, in press). The MLST site for *Xylella* (www.mlst.net) will be functional within the next six months (Scally pers. com). The genes used were chosen based on their informative pattern of evolution, and they are distributed throughout the *Xf* genome, summing to 9,307 base pairs. We are using the results of this project to characterize those strains we encounter in the riparian plants and in the BGSS, *Graphocephala atropunctata* (Signoret).

OBJECTIVES
1. Collect *Xf* samples from a diversity of native and naturalized introduced plants in and around the riparian zones in southern California
2. Collect *Xf* samples from adult BGSS
3. Characterize the *Xylella* strains that are recovered using MLST
4. Determine the associations between specific *Xf* strains, their plant hosts, and their geographic distributions

RESULTS
We have sampled a range of riparian plant species known to be host to BGSS or to harbor *Xf* (Purcell 1975, 1976) from riparian sites and sites near citrus groves and cultivated grapes in southern California. The plants in Table 1 were collected from October 2004 – August 2005. All of the plants listed as native are native to the continental United States, except those noted as being native to Central and South America. Xylem was extracted by the pressure bomb method. Plants were identified by botanist Andrew Sanders (UC, Riverside Herbarium) with reference to the Jepson Manual (Hickman 1993). Adult BGSS were collected from a riparian site in August of 2005; only the head was processed in order to avoid diluting the sample with additional insect DNA. DNeasy Plant or Tissue Extraction kits (Qiagen, Valencia, CA USA) were used to extract DNA. We performed real-time PCR using *Xf* specific ITS primers (*Xf*1) (Schaad et al. 2002) in order to determine the

presence or absence of the bacteria. The number of *Xf* positive and *Xf* negative samples for each plant are reported in Table 1. Additionally, we detected *Xf* in 4 out of 62 BGSS samples.

We have sequenced the two most variable MLST genes for each of the positive plant and insect samples. Strains that possess one or more genes that manifest a uniquely different sequence in any of these genes are treated as a different strain. Our preliminary results have revealed different *Xf* strains in common native plant species and in BGSS collected from the same location. We have identified the Pierce's disease strain in *Vitis girdiana* (wild grape), *Pluchea odorata* (sweetscent) and three BGSS from a riparian area in Temecula, California. The multiplex strain was identified from *Xanthium strumarium* (cocklebur), *Salvia mellifera* (black sage) and a BGSS collected in Temecula. Additionally, *Salvia mellifera* collected from Emerson Oaks Reserve and on the UC, Riverside campus also contained the multiplex strain. However, *Encelia farinosa* (brittlebush) collected in the Riverside area (on the UC, Riverside campus and in Two Trees Trail (a riparian area near the UC, Riverside campus) contained a new variant of the multiplex strain.

CONCLUSIONS

Until recently, the identified strains in California have been those associated with agricultural or ornamental host plants. We do not know how many asymptomatic indigenous strains exist in California, especially in native or naturalized alien plants because they have not, as yet, given rise to a recognizable syndrome. Our plant collection and xylem extraction efforts have targeted native and naturalized plants in riparian habitats that may harbor *Xf* which is spread from them to cultivated plants by infected BGSS. Additionally, the possibility of invasions by novel strains from other parts of the Americas cannot be ignored. Therefore, it is critically important that we characterize the diversity of *Xf* strains present in California, especially those presumed to be the ancestral strains, i.e., those in native and naturalized alien plant hosts. This information is essential for fully understanding the potential for recombination and the generation of new and potentially more virulent strains.

REFERENCES

Bextine, B., and T. A. Miller. 2005. Laboratory-based monitoring of an insect-transmitted plant pathogen system. BioTechniques 38: 184-186.

Enright, M. C., and B. G. Spratt. 1999. Multilocus sequence typing. Trends Microbiol. 7: 482-7.

Hickman, J. C. 1993. The Jepson Manual Higher Plants of California. Univ. of California Press. Berkeley, CA.

Maiden, M. C. J., J, A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I, M. Feavers, M. Achtman, and B. G. Spratt. 1998. Multilocus sequence typing: a portable approach to the identification of clones within population of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA. 95: 3140-45.

Oliveira, A. C., M. A. Vallim, C. P. Semighini, W. L. Araujo, G. H. Goldman, and M. A. Machado. 2002. Quantification of *Xylella fastidiosa* from citrus trees by real-time polymerase chain reaction assay. Phytopath. 92: 1048-1054.

Purcell, A. H. 1975. Role of blue-green sharpshooter, *Hordnia circellata* in the epidemiology of Pierce's disease of grapevines. Environ. Entomol. 4: 745-52.

Purcell, A. H. 1976. Seasonal changes in host plant preference of the blue-green sharpshooter, *Hordnia circellata*, (Homoptera: Cicadellidae). Pan-Pac. Entomol. 52: 33-7.

Scally, M., E.L. Schuenzel, R. Stouthamer, and L. Nunney. Estimating the relative contribution of recombination versus point mutation in the plant pathogen *Xylella fastidiosa* using MLST. Appl. Envir. Microbiol. (in press).

Schaad, N. W., D. Opgenorth, and P. Gaush. 2002. Real-time polymerase chain reaction for one-hour on-site diagnosis of Pierce's Disease of grape in early season asymptomatic vines. Phytopath. 70: 721-728.

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Table 1. Number of positive *Xf* and negative *Xf* plants sampled October 2004 - August 2005

Native	Family	+	-	Native	Family	+	-
Abutilon palmeri	Malvaceae	0	1	*Dudleya pulverulenta*	Crassulaceae	0	2
Adenostoma fasciculatum	Rosaceae	0	1	*Encelia californica*	Asteraceae	0	1
Amaranthus albus	Amaranthaceae	0	1	*Encelia farinosa*	Asteraceae	**5**	19
Ambrosia acanthacarpa	Asteraceae	0	1	*Epilobium ciliatum*	Onagraceae	0	1
Ambrosia confertiflora	Asteraceae	0	3	*Equisetum arvense*	Equisetaceae	0	2
Ambrosia psilostachya	Asteraceae	0	21	*Eriodictyon trichocalyx*	Hydrophyllaceae	0	2
Amorpha fruticosa	Fabaceae	0	2	*Eriogonum elongatum*	Polygonaceae	0	1
Amsinckia menziesii v. intermedia	Boraginaceae	0	3	*Eriogonum fasciculatum var. undet.*	Polygonaceae	0	4
Antirrhinum coulterianum	Scrophulariaceae	0	1	*Eriogonum fasciculatum v. polifolium*	Polygonaceae	0	5
Antirrhinum nuttallianum	Scrophulariaceae	0	2	*Eriogonum fasciculatum var. foliosum*	Polygonaceae	0	8
Apocynum cannabinum	Apocynaceae	0	7	*Eriophyllum confertiflorum*	Asteraceae	0	4
Araujia sericofera†	Asclepiadaceae	0	2	*Eucrypta chrysanthemifolia*	Hydrophyllaceae	0	6
Arctostaphylos sp.	Polygonaceae	0	2	*Fallugia paradoxa*	Rosaceae	0	1
Artemisia californica	Asteraceae	0	17	*Galium aparine*	Rubiaceae	0	1
Artemisia douglasiana	Asteraceae	0	33	*Gnaphalium californicum*	Asteraceae	0	1
Artemisia tridentata	Asteraceae	0	4	*Gnaphalium canescens ssp.microcephalum*	Asteraceae	0	7
Atriplex canescens	Chenopodiaceae	0	1	*Gnaphalium stramineum*	Asteraceae	0	1
Atriplex polycarpa	Chenopodiaceae	0	1	*Haplopappus squarrosus*	Asteraceae	0	2
Atriplex triangularis	Chenopodiaceae	0	3	*Helianthemum scoparium*	Cistaceae	0	1
Baccharis salicifolia	Asteraceae	0	17	*Helianthus annuus*	Asteraceae	0	5
Baccharis sergiloides	Asteraceae	0	2	*Heterotheca grandiflora*	Asteraceae	0	4
Berlandiera lyrata	Asteraceae	0	1	*Heteromeles arbutifolia*	Rosaceae	0	2
Bidens pilosa	Asteraceae	0	3	*Hyptis emoryi*	Lamiaceae	0	1
Brickellia sp.	Asteraceae	0	1	*Juncus rugulosus*	Juncaceae	0	2
Brickellia californica	Asteraceae	0	1	*Juniperus californica*	Cupressaceae	0	1
Calyptridium monandrum	Portulacaceae	0	1	*Justicia californica*	Acanthaceae	0	1
Camissonia bistorta	Onagraceae	0	1	*Justicia leonardii*	Acanthaceae	0	2
Camissonia brevipes	Onagraceae	0	1	*Helianthus gracilentus*	Asteraceae	0	2
Camissonia californica	Onagraceae	0	1	*Keckiella antirrhinoides*	Scrophulariaceae	0	1
Camissonia hirtella	Onagraceae	0	1	*Larrea tridentata*	Zygophyllaceae	0	1
Cardiospermum corindum	Sapindaceae	0	2	*Lathyrus vestitus*	Fabaceae	0	2
Ceanothus (greggii?)	Rhamnaceae	0	1	*Lavatera assurgentiflora*	Verbenaceae	0	1
Ceanothus tomentosus	Rhamnaceae	0	1	*Lessingia filaginifolia*	Asteraceae	0	1
Celtis reticulata	Ulmaceae	0	1	*Lippia wrightii*	Verbenaceae	0	2
Cercocarpus betuloides	Rosaceae	0	3	*Lotus heermannii*	Fabaceae	0	9
Chaenactis artemisiifolia	Asteraceae	0	1	*Lotus purshianus*	Fabaceae	0	1
Chamomilla suaveolens	Asteraceae	0	1	*Lotus scoparius var. brevialatus*	Fabaceae	0	11
Chenopodium berlandieri	Chenopodiaceae	0	3	*Lotus strigosus*	Fabaceae	0	1
Chilopsis linearis	Bignoniaceae	0	2	*Lupinus bicolor*	Fabaceae	0	2
Chrysothamnus nauseosus	Asteraceae	0	1	*Lupinus truncatus*	Fabaceae	0	1
Conyza canadensis	Asteraceae	0	9	*Malacothamnus sp.*	Malvaceae	0	1
Croton californicus	Euphorbiaceae	0	2	*Malacothamnus fasciculatus*	Malvaceae	0	2
Cryptantha intermedia	Boraginaceae	0	1	*Malosma laurina*	Anacardiaceae	0	8
Cryptantha muricata	Boraginaceae	0	4	*Marah macrocarpus*	Cucurbitaceae	0	6
Cyperus eragrostis	Cyperaceae	0	2	*Mentha arvensis*	Lamiaceae	0	1
Datura wrightii	Solanaceae	0	5	*Mirabilis californica*	Nyctaginaceae	0	1
Delphinium cardinale	Ranunculaceae	0	1	*Mimulus aurantiacus*	Scrophulariaceae	0	8
Dryopteris arguta	Dryopteridaceae	0	2	*Mimulus aurantiacus var. puniceus*	Scrophulariaceae	0	8

Table 1 continued

Native	Family	+	-	Naturalized	Family	+	-
Mimulus brevipes	Scrophulariaceae	0	1	*Anagallis arvensis*	Primulaceae	0	2
Mimulus cardinalis	Scrophulariaceae	0	5	*Apium graveolens*	Apiaceae	0	3
Nicotiana glauca†	Solanaceae	0	18	*Erosa saracophera*	Asclepiadaceae	0	2
Oenothera elata	Onagraceae	0	3	*Brassica geniculata*	Brassicaceae	0	8
Parkinsonia aculeata	Fabaceae	0	2	*Chenopodium murale*	Chenopodiaceae	0	2
Penstemon spectabilis	Scrophulariaceae	0	1	*Chenopodium ambrosioides*	Chenopodiaceae	0	7
Plantago major	Plantaginaceae	0	5	*Cirsium vulgare*	Asteraceae	0	1
Platanus racemosa	Platanaceae	0	16	*Cnicus benedictus*	Asteraceae	0	1
Pluchea odorata	Asteraceae	**1**	1	*Cirsium vulgare*	Asteraceae	0	1
Polygonum lapathifolium	Polygonaceae	0	9	*Cnicus benedictus*	Asteraceae	0	1
Prunus caroliniana	Rosaceae	0	3	*Conyza floribunda*	Asteraceae	0	2
Prunus fasciculata	Rosaceae	0	2	*Cotula australis*	Asteraceae	0	1
Prunus virginia	Rosaceae	0	2	*Cyperus involucratus*	Cyperaceae	0	3
Phacelia minor	Hydrophyllaceae	0	1	*Eremocarpus setigerus*	Euphorbiaceae	0	2
Phacelia distans	Hydrophyllaceae	0	2	*Erodium cicutarium*	Geraniaceae	0	3
Phacelia ramosissima	Hydrophyllaceae	0	6	*Erodium moschatum*	Geraniaceae	0	2
Prosopis (glandulosa?)	Fabaceae	0	2	*Euphorbia peplis*	Euphorbiaceae	0	1
Psoralea macrostachya	Fabaceae	0	1	*Foeniculum vulgare*	Apiaceae	0	1
Pteridium aquilinum	Dennstaedtiaceae	0	1	*Gnaphalium luteo-album*	Asteraceae	0	3
Purshia neomexicana	Rosaceae	0	1	*Lactuca serriola*	Asteraceae	0	3
Quercus agrifolia	Fagaceae	0	1	*Lonicera japonica*	Caprifoliaceae	0	2
Ribes diverticatum	Grossulariacea	0	1	*Lonicera subspicata*	Caprifoliaceae	0	1
Robinia neomexicana	Fabaceae	0	1	*Malva parviflora*	Malvaceae	0	5
Romneya coulteri	Papaveraceae	0	2	*Marrubium vulgare*	Lamiaceae	0	5
Rorippa nasturtium-aquaticum	Brassicaceae	0	2	*Melilotis alba*	Fabaceae	0	3
Rosa californica	Rosaceae	0	8	*Mentha suaveolens*	Lamiaceae	0	3
Rubus ursinus	Rosaceae	0	9	*Metrosideros sp*	Myrtaceae	**2**	2
Rumex salicifolius	Polygonaceae	0	2	*Metrosideros kermadecensis*	Myrtaceae	0	6
Rubus parviflorus	Rosaceae	0	2	*Nerium oleander*	Apocynaceae	**1**	1
Salix lasiolepis	Salicaceae	0	18	*Oxalis rubra*	Oxalidaceae	0	1
Salix laevigata	Salicaceae	0	8	*Picris echioides*	Asteraceae	0	4
Salvia apiana	Lamiaceae	0	13	*Plantago lanceolata*	Plantaginaceae	0	1
Salvia mellifera	Lamiaceae	**3**	21	*Prunus dulcis*	Rosaceae	0	1
Sambucus mexicana	Caprifoliaceae	0	6	*Phragmites australis*	Poaceae	0	1
Samolus parviflorus	Primulaceae	0	1	*Ricinus communis*	Euphorbiaceae	0	2
Sanicula crassicaulis	Apiaceae	0	1	*Raphanus sativus*	Brassicaceae	0	1
Scrophularia californica	Scrophulariaceae	0	1	*Rhaphiolepis sp.*	Rosaceae	0	2
Stachys ajugoides	Lamiaceae	0	12	*Rhaphiolepis indica*	Rosaceae	0	5
Solanum americanum	Solanaceae	0	1	*Rumex crispus*	Polygonaceae	0	1
Solanum douglasii	Solanaceae	0	4	*Rumex conglomeratus*	Polygonaceae	0	1
Solanum xanti	Solanaceae	0	1	*Rumex salicifolias*	Polygonaceae	0	1
Symphoricarpos sp.	Caprifoliaceae	0	1	*Silybum marianum*	Asteraceae	0	1
Typha domingensis	Typhaceae	0	1	*Sisymbrium erysimoides*	Brassicaceae	0	2
Urtica dioica	Urticaceae	0	4	*Salsola tragus*	Chenopodiaceae	0	1
Venegasia carpesioides	Asteraceae	0	1	*Sisymbrium irio*	Brassicaceae	0	6
Vitis girdiana	Vitaceae	**1**	27	*Sonchus oleraceus*	Asteraceae	0	1
Xanthium strumarium	Asteraceae	**1**	29	*Vinca major*	Apocynaceae	0	3

† native to Central or South America