# A systematic study of Xylella fastidiosa strains isolated from pecan, grapevine, oleander, and sycamore in Louisiana 

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# A SYSTEMATIC STUDY OF XYLELLA FASTIDIOSA STRAINS ISOLATED FROM PECAN, GRAPEVINE, OLEANDER, AND SYCAMORE IN LOUISIANA 

## A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of Master of Science
in
The Department of Plant Pathology \& Crop Physiology
by
Rebecca Ann Melanson
B. S., Centenary College of Louisiana, 2003

December 2011

## DEDICATION

I dedicate this thesis to my parents, Ulysses T. Melanson and Kay L. Melanson, who began educating me as a child and have encouraged me to continue my education ever since. They have relentlessly supported my efforts and given themselves in any means necessary to help me on my journey. I will always be grateful.

## ACKNOWLEDGEMENTS

I would like to thank my parents, Ulysses T. Melanson and Kay L. Melanson, for being a constant support throughout my journey. Your support and encouragement have helped give me the confidence and strength necessary to travel the journey.

I would like to thank my advisor, Dr. Jong Hyun Ham, for accepting me as a graduate student and for guiding me and pushing me on my path to becoming a better researcher, plant pathologist, and professional.

I would like to thank my committee members, Dr. Zhi-Yuan Chen, Dr. Christopher A. Clark, Dr. Gregg Pettis, and Dr. Rodrigo A. Valverde, for the guidance and support they have given me in pursuing a career as a researcher and as a plant pathologist.

I would like to thank Dr. Randy S. Sanderlin for introducing me to the world of plant pathology and for being my first mentor in the field and a source of continuous support.

I would like to thank Dr. Donald Ferrin, Dr. Marc A. Cohn, Dr. Jere McBride, Dr. Lawrence Datnoff, Dr. Alistair McTaggart, Dr. Charles Overstreet and the other members of the Department of Plant Pathology and Crop Physiology for all the advice, assistance, and support that they have given me.

I would like to thank the Louisiana State University Graduate School, the Louisiana State University Agricultural Center and Dr. Raghuwinder Singh and Dr. Donald Ferrin for providing funding to attend Louisiana State University, to complete the work described in this thesis, and for the use of the strain of $X$. fastidiosa from oleander in Louisiana, respectively.

Finally, I would like to thank my family, friends, lab mates, co-workers, and fellow students for putting up with me, for sharing in my frustration and excitement, and for encouraging me and helping me along the way, especially Bishnu K. Shrestha, Dr. Nicole A.

Ward, and Ashok Chanda from the Department of Plant Pathology and Crop Physiology. The rest of you know who you are and your friendship and support will not be forgotten.

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#### Abstract

Xylella fastidiosa causes disease in a number of economically important crops and landscape shrubs and trees including grapevine, citrus, oleander, and sycamore. In pecan, $X$. fastidiosa causes pecan bacterial leaf scorch (PBLS), which leads to defoliation and reduces nut yield. No economically effective treatments are available for PBLS. In order to improve PBLS management practices, it is necessary to determine the subspecies of $X$. fastidiosa strains that infect pecan so potential sources of inoculum can be identified. Multiprimer PCR and phylogenetic analyses using nucleotide sequence data from the $16 \mathrm{~S}-23 \mathrm{~S}$ rDNA intergenic transcribed spacer (ITS) region and pglA consistently identified strains of $X$. fastidiosa isolated from pecan as $X$. fastidiosa subsp. multiplex. Enterobacterial repetitive intergenic consensus (ERIC)-PCR and repetitive extragenic palindromic (REP)-PCR analyses were congruent with phylogenetic analyses. REP-PCR analyses indicated genetic variation within strains of $X$. fastidiosa from pecan. From these same analyses, X. fastidiosa strains from sycamore, grapevine and oleander from Louisiana were identified as subsp. multiplex, subsp. fastidiosa and subsp. sandyi, respectively. This study provides additional information about the host ranges of $X$. fastidiosa subspecies.


## CHAPTER 1: INTRODUCTION

## LITERATURE REVIEW

The Plant Pathogen, Xylella fastidiosa. X. fastidiosa is a xylem-limited, fastidious bacterium that causes disease in a number of economically important crops, ornamentals, and shade trees, including grapevine, peach, citrus, oleander, and sycamore (Chang et al., 1993; Davis et al., 1978; Purcell et al., 1999; Sherald et al., 1983; Wells et al., 1987). The pathogen is spread from infected plant hosts to non-infected plant hosts by graft transmission and by xylemfeeding insects (Chatterjee et al., 2008; Redak et al., 2004; Sanderlin, 2005). Prior to 1987, some of the diseases now attributed to $X$. fastidios $a$ were recognized, but the causal agent was thought to be a virus or was described simply as a rickettsia-like organism or a xylem-limited bacterium (Hopkins et al., 1973; Hopkins and Mollenhauser, 1973; Sherald et al., 1983). It was not until the development of specialized media to culture the pathogen from infected hosts and the work of Wells et al. (1987) that xylem-limited bacteria producing similar diseases and showing similarity to each other but distinction from other genera were assigned to the new genus and species (Davis et al., 1978; Wells et al., 1987; Wells et al., 1981).
X. fastidiosa are Gram-negative, straight, rod-shaped cells $0.25-0.35 \times 0.9-3.5 \mu \mathrm{~m}$ in width and length (Brenner, 2005). They are aerobic and aflagellate, grow optimally from 26$28^{\circ} \mathrm{C}$, and have a GC content, as determined by melting temperature, of $51.0-52.4 \%$ (Brenner, 2005). These bacteria are nutritionally fastidious, requiring specialized media for growth in the laboratory, and a number of different media have been developed for culturing of $X$. fastidiosa (Brenner, 2005; Schaad, 2001).

In 2000, a citrus variegated chlorosis (CVC) strain of $X$. fastidiosa became the first $X$. fastidiosa strain to be completely sequenced, as well as the first plant pathogenic bacterium to be completely sequenced (Simpson et al., 2000). Since then, a number of other strains have been
sequenced including two strains from grapevine, Temecula1 and XF GB514, and two strains from almond, M12 and M23 (Chen et al., 2010; Schreiber IV et al., 2010; Van Sluys et al., 2003). The genomes of these strains range in size from approximately 2.48 Mb for almond strain M12 to 2.68 Mb for CVC strain 9 a 5 c ; one or two plasmids are also present in some strains (Chen et al., 2010; Schreiber IV et al., 2010; Simpson et al., 2000; Van Sluys et al., 2003). The complete genome sequences with gene annotations for these five strains are available in the National Center for Biotechnology Information's (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/). A gapped genome sequence of an oleander strain has also been determined (Bhattacharyya et al., 2002).

Even before the designation of the new genus and species in 1987, researchers noticed differences among isolates of various hosts and proposed that different groups or pathovars existed (Kamper et al., 1985). Since then, a number of subspecies have been proposed. Three subspecies of $X$. fastidiosa were proposed in 2004 based on DNA-DNA relatedness tests and intergenic transcribed spacer (ITS) sequence comparisons; the proposed subspecies were $X$. fastidiosa subsp. piercei, X. fastidiosa subsp. multiplex, and X. fastidiosa subsp. pauca (Schaad et al., 2004b). An erratum published after the original paper correctly renamed the proposed $X$. fastidiosa subspecies piercei to $X$. fastidiosa subspecies fastidiosa after the designated type strain of the species (Schaad et al., 2004a; Wells et al., 1987). Subspecies fastidiosa includes strains causing disease in alfalfa, almond, grapevine, and maple; subspecies multiplex includes strains causing disease in almond, elm, peach, pigeon grape, plum, sycamore, and other shade trees; and subspecies pauca includes stains causing disease in citrus (Schaad et al., 2004b). Two other subspecies of $X$. fastidiosa have since been proposed for strains infecting other hosts. $X$. fastidiosa subsp. sandyi was proposed for strains infecting oleander and is now known to infect
some other hosts (Hernandez-Martinez et al., 2007; Schuenzel et al., 2005). Recently, a fifth subspecies, $X$. fastidiosa subsp. tashke, was proposed for strains of $X$. fastidiosa infecting chitalpa in the southwestern United States (Randall et al., 2009).

With the exception of a pear disease caused by $X$. fastidiosa in Taiwan (Leu and Su , 1993) and a report of $X$. fastidiosa being isolated from a grapevine in Europe (Berisha et al., 1998), X. fastidiosa is limited to the North and South American continents (Hopkins and Purcell, 2002). Of the five described subspecies, X. fastidiosa subsp. pauca occurs only in South America, while the remaining subspecies are known to occur in North America (Randall et al., 2009; Schaad et al., 2004a; Schaad et al., 2004b; Schuenzel et al., 2005). Recent genomic studies suggest that $X$. fastidiosa subsp. fastidiosa and $X$. fastidiosa subsp. sandyi were introduced into the United States from more tropical regions, whereas $X$. fastidiosa subsp. multiplex evolved in a temperate region (Nunney et al., 2010). While the center of origin of $X$. fastidiosa subsp. sandyi is not yet known, evidence points to Central America as the center of origin of $X$. fastidiosa subsp. fastidiosa and North America as the source of $X$. fastidiosa subsp. multiplex (Nunney et al., 2010).

The study of $X$. fastidiosa is becoming increasingly important as new diseases caused by this pathogen are reported and new subspecies are identified. These events are possible and new diseases and subspecies are easier to identify and report as advancements in technology continue and as information about this species increases. Among the techniques used to study $X$. fastidiosa, the polymerase chain reaction (PCR), which has gained much popularity since its development, is routinely used in laboratory investigations. Perhaps the most basic of its uses is to detect the presence of the pathogen in plant tissue or to identify a particular strain of the pathogen. In 1994, PCR with the use of $X$. fastidiosa-specific primers was developed to detect $X$.
fastidiosa in plant tissues (Minsavage et al., 1994). The following year, primers specific for strains of X. fastidiosa causing CVC were developed (Pooler and Hartung, 1995). Further illustrating the use of PCR and the resourcefulness of published sequences, primers were developed for a multiprimer PCR that produced banding patterns unique to different $X$. fastidiosa genotypes (Almeida and Purcell, 2003; Hernandez-Martinez et al., 2006).

Another application of PCR that has been used in studies of $X$. fastidiosa is repetitive sequence-based PCR (rep-PCR). This technique uses the presence of repetitive elements, such as repetitive element palindromic (REP) sequences, enterobacterial repetitive intergenic consensus (ERIC) sequences, and BOX sequences, located in various positions throughout bacterial genomes to amplify different numbers and sizes of DNA bands that are unique for different bacterial species and strains and create a DNA fingerprint for each species or strain (Versalovic et al., 1991; Versalovic et al., 1994). The application of rep-PCR to examine the presence of REP and ERIC elements in the genomes of strains of $X$. fastidiosa from grapevine, maple, almond, and oak showed that, with the exception of the strain from maple that had the same fingerprint as the strains from grapevine in California, strains from the same host had the same rep-PCR fingerprints, but were different from those of other hosts (Hendson et al., 2001). In addition, strains of $X$. fastidiosa from grapevine in California and in the southeastern United States had the same ERIC-PCR fingerprint (Hendson et al., 2001). In another study, strains of $X$. fastidiosa from citrus growing in different geographic regions in Brazil had different fingerprints and could be differentiated from X. fastidiosa strains from plum, coffee, and pear (Mehta et al., 2001).

While a number of methods, including restriction fragment length polymorphism (RFLP) and rep-PCR, have been used to study the relationships among strains of $X$. fastidiosa isolated
from various hosts, PCR and phylogenetic analysis of nucleotide sequences are being used with increasing frequency to differentiate strains and to determine strain relatedness (Chen et al., 1992; Doddapaneni et al., 2007; Schaad et al., 2004b). The 16S rDNA region is a highly conserved region of DNA. It has been shown to be useful in identifying X. fastidiosa from other bacterial species and was used in the naming of the new genus (Chen et al., 2000a; Wells et al., 1987). Since then, the 16 S rDNA sequence has been used to compare strains of $X$. fastidiosa from a number of hosts (Chen et al., 2000b; Mehta and Rosato, 2001). The less conserved 16S23S ITS region has also been used as a means of strain comparison, often in conjunction with other tests or DNA sequences (Hendson et al., 2001; Martinati et al., 2005; Mehta and Rosato, 2001; Randall et al., 2009). Phylogenetic analysis of the 16S-23S ITS region, for example, was used to test the patterns observed for strains in the multiprimer PCR, and indeed, corresponded favorably with the observed patterns (Hernandez-Martinez et al., 2006). Sequences from both the 16 S rDNA and the $16 \mathrm{~S}-23 \mathrm{~S}$ ITS regions from these studies are available in NCBI's GenBank.

Multilocus sequence typing (MLST) systems for comparison of bacterial strains are emerging as a popular technique. MLST analyses use partial DNA sequences from a set of housekeeping genes that encode necessary bacterial functions and that are unique for the particular group of bacteria being studied to characterize and group those bacteria (Maiden, 2006). Partial sequence data for a number of housekeeping genes, including holC, $r f b D$, nuoL, nuoN, gltT, cysG, petC, pilU, leuA, and lacF (later renamed malF), were obtained from $X$. fastidiosa strains and then subjected to phylogentic analysis to study the geographic variation and evolutionary relationships among strains of X. fastidiosa (Schuenzel et al., 2005; Yuan et al., 2010). Seven of these ten genes were used in an MLST analysis that resulted in the differentiation of $X$. fastidiosa strains into clonal complexes (correlated to subspecies) and
sequence types within clonal complexes (Scally et al., 2005). Since the original MLST analysis, primers to amplify portions of these seven genes were redesigned for better amplification of products or for amplification of gene products from X. fastidiosa subsp. pauca, as well as shorter products that would result in better sequence reads (Yuan et al., 2010). Results from the revised MLST analysis showed that little genetic variation existed within X. fastidiosa subsp. fastidiosa and X. fastidiosa subsp. sandyi (Yuan et al., 2010). In addition to characterizing and grouping closely related bacterial strains, MLST analysis also has the ability to detect recombination (Spratt et al., 2001). This is evident in the X. fastidiosa MLST system as both MLST systems, using the original primers and the revised primers, showed genetic recombination between $X$. fastidiosa subsp. fastidiosa and $X$. fastidiosa subsp. multiplex in the cysG, holC, and pilU genes of some strains (Scally et al., 2005; Yuan et al., 2010). In addition to the recombination seen between X. fastidiosa subsp. fastidiosa and X. fastidiosa subsp. multiplex (Scally et al., 2005; Yuan et al., 2010), MLST analyses have also shown recombination between strains of $X$. fastidiosa subsp. pauca infecting coffee and citrus (Almeida et al., 2008). A MLST database for X. fastidiosa typing is available at http://pubmlst.org/xfastidiosa/.

A number of potential virulence products and their encoding genes, such as $p g l A$ and $v a p D$, have been described in $X$. fastidiosa and are being examined to determine their presence or absence among particular strains (Marques et al., 2001; Simpson et al., 2000). Polygalacturonase (PglA) is required for colonization and pathogenicity in grapevine (Roper et al., 2007). In X. fastidiosa CVC and coffee strains, pglA, which encodes a polygalacturonase precursor, has early termination as a result of a frameshift; this frameshift was not present in the almond, grapevine, and mulberry strains (Van Sluys et al., 2003). A gene encoding a virulenceassociated protein D homologue, vapD, was identified on the pXF51 plasmid of the CVC 9a5c
strain (Marques et al., 2001). A putative $v a p D$ gene was also identified in the Ann-1 oleander strain (Randall et al., 2009). The vapD sequence was cloned from strains of $X$. fastidiosa from chiltalpa and sequenced, and nucleotide and protein sequences were compared using NCBI's Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990; Randall et al., 2009).

Pecan Bacterial Leaf Scorch. In 2000, X. fastidiosa was reported as the cause of a leaf scorch disease of pecan (Carya illinoinensis) previously attributed to fungi, and it was proposed that the disease name be changed from fungal leaf scorch to pecan bacterial leaf scorch (PBLS) (Sanderlin and Heyderich-Alger, 2000). The symptoms of PBLS are similar to those caused by the bacterium in some other plant hosts and include leaflets turning tan to brown beginning at the margin and progressing basipetally, followed by abscission of affected leaflets (Sanderlin and Heyderich-Alger, 2000). In a study on cultivar Cape Fear pecan trees, PBLS diseased trees had reduced kernel weights averaging $16 \%$ when compared to non-infected trees in the same orchard (Sanderlin and Heyderich-Alger, 2003). PBLS has been identified in over 20 pecan cultivars and in seedling trees (Sanderlin, 2005; Sanderlin and Melanson, 2007).

The majority of the studies involving $X$. fastidiosa in pecan have focused on the transmission of the pathogen in pecan. $X$. fastidiosa has been shown to survive the typical scion wood storage for pecan and can be transmitted from scions of infected trees into newly developing trees following grafting (Sanderlin, 2005). Likewise, the pathogen is also able to pass from infected pecan rootstock, through the graft union, and into scions collected from noninfected pecan (Sanderlin and Melanson, 2006). Graft transmission of the pathogen may be a major source of spread into pecan orchards (Sanderlin, 2005). A method of treating pecan scion wood with hot water successfully reduces graft transmission of the pathogen (Sanderlin and Melanson, 2008). However, the pathogen continues to spread in established orchards, apparently
by insect transmission (Li et al., 2011; Sanderlin et al., 2009). Insect transmission tests with the pecan spittlebug, Clastoptera achatina, and several leafhopper species, including Homalodisca vitripennis and $H$. insolita, have shown that the pathogen can also be transmitted by some insects from infected to non-infected pecan and cause disease (Melanson et al., 2009; Sanderlin et al., 2009).

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# CHAPTER 2: A SYSTEMATIC STUDY OF THE 16S-23S RDNA INTERGENIC TRANSCRIBED SPACER REGION, PGLA, AND ERIC-PCR AND REP-PCR FINGERPRINTS REVEALS THAT XYLELLA FASTIDIOSA STRAINS FROM PECAN ARE PART OF $X$. FASTIDIOSA SUBSP. MULTIPLE ${ }^{1}$ 

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## INTRODUCTION

Xylella fastidiosa is a Gram-negative, xylem-limited, fastidious bacterium that causes disease in a number of economically important crops, ornamental plants, and shade trees, including grapevine, peach, citrus, oleander, and sycamore (Chang et al., 1993; Davis et al., 1978; Purcell et al., 1999; Sherald et al., 1983; Wells et al., 1987). Symptoms of disease in these hosts typically include scorching of leaves, reduced growth, defoliation, plant death, or chlorosis (Chen et al., 2005; Hopkins and Purcell 2002; Purcell et al., 1999; Sanderlin and HeyderichAlger 2000; Sherald et al., 1983; Simpson, et al., 2000). Insect transmission by xylem-feeding insects in the families Cercopidae and Cicadellidae is considered the primary means of transmission of $X$. fastidiosa from infected to non-infected plant hosts (Redak et al., 2004); however, other forms of transmission, including graft transmission and seed transmission, have also been reported in some hosts (Chatterjee et al., 2008; Li, W. B. et al., 2003; Redak et al., 2004; Sanderlin, 2005).

Five subspecies of $X$. fastidiosa have been determined since the genus and species was first described by Wells, et al. in 1987 (Randall et al., 2009; Schaad et al., 2004a; Schaad et al., 2004b; Schuenzel et al., 2005; Wells et al., 1987). These include X. fastidiosa subsp. fastidiosa, which infects and causes disease in grapevine and almond, as well as in other plant hosts (Schaad et al., 2004a; Schaad et al., 2004b); X. fastidiosa subsp. pauca, which infects citrus and coffee and is the only described subspecies not present in North America (Nunney et al., 2010; Schaad et al., 2004b); X. fastidiosa subsp. multiplex, which infects a large number of plant hosts, including almond, elm, peach, pigeon grape, plum, sycamore, and other shade trees (Schaad et al., 2004b); X. fastidiosa subsp. sandyi, which was described for those strains infecting oleander and some other hosts (Schuenzel et al., 2005); and the most recently described subspecies, $X$.
fastidiosa subsp. tashke, which contains strains infecting chitalpa in the southwestern United States (Randall et al., 2009). Some degree of host specificity exists among subspecies and strains of $X$. fastidiosa; however, this specificity is not always clear and some subspecies and strains are able to infect multiple plant hosts (Hopkins, 1989). Plant hosts, including grapevine and sycamore, growing in Louisiana have been observed with their respective X. fastidiosainduced diseases, and recently, X. fastidiosa causing oleander leaf scorch was reported in Louisiana (Singh et al., 2010).
X. fastidiosa 9a5c, a strain isolated from citrus (sweet orange), was the first plant pathogenic bacterium to have its genome completely sequenced (Simpson et al., 2000). Since then, additional strains of $X$. fastidiosa, including strains from almond and grapevine, have been completely sequenced; sequence information for these whole genomes as well as many DNA regions and genes of other strains is available in the National Center for Biotechnology Information's (NCBI) GenBank database (Chen et al., 2010; Schreiber IV et al., 2010; Van Sluys et al., 2003). The availability of these complete genome sequences, as well as other sequence information from the 16 S rDNA, the $16 \mathrm{~S}-23 \mathrm{~S}$ rDNA ITS region, and various genes, has enabled comparative genomic/genetic analyses among strains from different hosts and subspecies and has led to assays for the differentiation of subspecies as well as the identification of new subspecies (Hernandez-Martinez et al., 2006; Huang, 2009; Schuenzel et al., 2005).

In 2000, X. fastidiosa was reported as the causative agent of pecan (Carya illinoinensis (Wangenheim) K. Koch) bacterial leaf scorch (PBLS) (Sanderlin and Heyderich-Alger, 2000). The symptoms of PBLS are similar to those caused by the bacterium in other plant hosts and include leaflets turning tan to brown beginning at the margin and progressing basipetally, followed by abscission of infected leaflets (Sanderlin and Heyderich-Alger, 2000). This disease
tends to occur annually and has been identified in over 20 pecan cultivars and in seedling trees (Sanderlin, 2005; Sanderlin and Melanson, 2007). X. fastidiosa threatens the pecan industry by causing reduced nut yields in infected trees. In pecan cv. Cape Fear trees, diseased terminals had reduced kernel weights that averaged $16 \%$ less when compared to non-infected terminals in the same orchard (Sanderlin and Heyderich-Alger, 2003). Currently, only management strategies to reduce or eliminate the introduction of this pathogen into pecan orchards exist. No resistant pecan cultivars have been identified, and there is no treatment for trees that become infected.

Compared to other diseases caused by X. fastidiosa, PBLS is a recently recognized disease. The majority of the studies on PBLS have been focused on the transmission of the pathogen in pecan. Because pecan cultivars are clonally propagated, graft transmission of the pathogen may be a major source of pathogen spread into orchards. X. fastidiosa can be transmitted from either scions or rootstocks obtained from infected pecan trees following grafting (Sanderlin, 2005; Sanderlin and Melanson, 2006). A hot-water treatment of pecan scion wood reduced graft transmission of the pathogen; however, the pathogen continues to spread in established orchards, apparently by insect transmission (Li, B. et al., 2011; Sanderlin et al., 2009; Sanderlin and Melanson, 2008). Most recently, insect transmission tests in pecan have shown that the pecan spittlebug, Clastoptera achatina, and several leafhopper species, including the glassy-winged sharpshooter (GWSS), Homalodisca vitripennis, and the Johnsongrass sharpshooter, H. insolita, are able to transmit X. fastidiosa from infected to non-infected pecan with subsequent PBLS development (Sanderlin and Melanson, 2010).

Thus far, pecan strains of $X$. fastidiosa have not been taxonomically grouped at the subspecies level. The taxonomic identification of the pecan strains, as well as their relationship to other strains of $X$. fastidiosa present in Louisiana and in other hosts, would aid in the
development of more effective management strategies for PBLS. These strategies should take into account other plant hosts in and around pecan orchards that may harbor the same subspecies and serve as sources of inoculum for transmission by insect vectors, such as the GWSS, which feed on and transmit the pathogen in several hosts (Adlerz and Hopkins, 1979; Almeida and Purcell, 2003b; Damsteegt et al., 2006; Leininger, 2004; Purcell et al., 1999).

This study determined the subspecies of $X$. fastidiosa strains isolated from pecan and other hosts in Louisiana and the phylogenetic relationship among the strains using a multiprimer PCR assay, DNA sequence analyses, and rep-PCR techniques. The information obtained through this study will contribute to a better understanding of PBLS epidemiology and X. fastidiosa population biology.

## MATERIALS AND METHODS

Bacterial Strains, Isolation, Growth Conditions, and DNA Extraction. X. fastidiosa strains used in this study are listed in Table 1. Bacterial strains were previously isolated from plant tissue showing scorch-like symptoms by squeezing plant sap from pecan rachises and from grapevine and sycamore petioles directly onto a periwinkle wilt medium (Davis et al., 1981) modified by D. L. Hopkins (Sanderlin, personal communication). Culture plates were subsequently incubated in the dark at $28^{\circ} \mathrm{C}$ and were examined periodically. Individual colonies resembling $X$. fastidiosa were propagated, transferred to tubes containing $30 \%$ glycerol, and stored at -60 to $-70^{\circ} \mathrm{C}$. The strain from oleander was isolated as previously described (Singh et al., 2010).

Strains from pecan, grapevine, and sycamore previously tested positive for $X$. fastidiosa with a commercial X. fastidiosa DAS-ELISA kit (Agdia, Inc., Elkhart, IN). The oleander strain, XF Oleander, was previously verified as $X$. fastidiosa using primers specific for $X$. fastidiosa
oleander strains. (Huang, 2009; Singh et al., 2010). All strains were recovered from glycerol storage and grown on modified periwinkle wilt medium at $28^{\circ} \mathrm{C}$. Genomic DNA was extracted from 11-day-old cultures of all pecan, grapevine, and oleander strains, and from a one-month-old culture of the sycamore strain using the GenElute Bacterial Genomic DNA Extraction kit (Sigma, St. Louis, MO). DNA samples were stored at $-20^{\circ} \mathrm{C}$ for long-term use or $4^{\circ} \mathrm{C}$ for shortterm use.

Table 1. Strains of Xylella fastidiosa Isolated from Symptomatic Plant Tissue and Used in This Study

| Strain designation | Host plant (cultivar) ${ }^{\text {a }}$ | Location of plant host ${ }^{\text {b }}$ | Year of isolation |
| :---: | :---: | :---: | :---: |
| XF A-05 | Carya illinoinensis | Shreveport, LA ${ }^{\text {c }}$ | 2005 |
|  | Pecan (Cape Fear) |  |  |
| XF B-05 | C. illinoinensis | Shreveport, LA ${ }^{\text {c }}$ | 2005 |
|  | Pecan (Cape Fear) |  |  |
| XF C-05 | C. illinoinensis | Shreveport, LA ${ }^{\text {c }}$ | 2005 |
|  | Pecan (Cape Fear) |  |  |
| XF A-06 | C. illinoinensis | Shreveport, LA ${ }^{\text {c }}$ | 2006 |
|  | Pecan (Cape Fear) |  |  |
| XF A-07 | Platanus occidentalis | Shreveport, LA | 2007 |
|  | Sycamore |  |  |
| XF D-07 | C. illinoinensis | Shreveport, LA ${ }^{\text {c }}$ | 2007 |
|  | Pecan (Cape Fear) |  |  |
| XF A-08 | Vitis vinifera | Hessmer, LA ${ }^{\text {d }}$ | 2008 |
|  | Grapevine |  |  |
| XF Oleander | Nerium oleander | Baton Rouge, LA | 2009 |
|  | Oleander |  |  |
| XF A-10 | C. illinoinensis | Hessmer, LA ${ }^{\text {d }}$ | 2010 |
|  | Pecan (Oconee) |  |  |
| XF B-10 | C. illinoinensis | Hessmer, LA ${ }^{\text {d }}$ | 2010 |
|  | Pecan (Desirable) |  |  |

${ }^{a}$ The scientific name, common name, and cultivar (in parentheses) for host plant are given whenever the information is known.
${ }^{\mathrm{b}}$ Shreveport, LA, is located in Caddo Parish; Hessmer, LA, is located in Avoyelles Parish; Baton Rouge, LA, is located in East Baton Rouge Parish.
${ }^{\text {c }}$ Strains were isolated from symptomatic orchard trees or trees in pots at the Pecan ResearchExtension Station in Shreveport, LA.
${ }^{\mathrm{d}}$ Strains were isolated from symptomatic trees or vines growing at the same location in Hessmer, LA.
$\boldsymbol{X}$. fastidiosa-specific PCR. Standard PCR was performed on all strains of $X$. fastidiosa from Louisiana with primers RST31 and RST33 (Table 2) to confirm previous ELISA and PCR results. The PCR cycling parameters and procedure and electrophoresis conditions are described in Table 3. PCR was repeated at least twice for each bacterial strain.

Multiprimer PCR Assay. A multiprimer PCR assay designed by Hernandez-Martinez, et al. (2006) was performed on all strains of $X$. fastidiosa from Louisiana. Primers used in the multiprimer PCR assay are listed in Table 2. The PCR cycling parameters and procedure and electrophoresis conditions are described in Table 3. PCR was repeated at least twice for each bacterial strain.

PCR and Sequence Analysis of the 16S-23S rDNA Intergenic Transcribed Spacer
(ITS) Region and $\boldsymbol{p g l a}$. Primers G1 and L1 (Table 2) were used to amplify the 16S-23S rDNA ITS region of strains of $X$. fastidiosa. The PCR cycling parameters and procedure, modified from Hendson et al. (2001), are described in Table 3.

Primers XFPglA_Fw and XFPglA_Rv (Table 2) were designed to amplify the pglA gene of strains of $X$. fastidiosa. The PCR cycling parameters and procedure are described in Table 3.

Aliquots of the ITS and pglA PCR products were tested by gel electrophoresis as described in Table 3 to confirm PCR product. The remaining ITS and pglA PCR products were purified using the QuickClean 5M PCR Purification Kit (GenScript, Piscataway, NJ). Purified PCR products were sequenced by the Louisiana State University School of Veterinary Medicine's GeneLab, Baton Rouge, LA, or by Macrogen Inc., Seoul, Korea. Products from at least two independent PCR reactions for ITS and $p g l A$ were sequenced for each strain to ensure correct base identification with the exception of strains XF A-10 and XF B-10 from pecan whose ITS sequence was not obtained or was determined from a single PCR product, respectively

Table 2. Primers ${ }^{\text {a }}$ Used in This Study

| Primer name | Primer sequence ( $5^{\prime} \rightarrow 3^{\prime}$ ) | Test | Reference |
| :---: | :---: | :---: | :---: |
| RST31 | GCGTTAATTTTCGAAGTGATTCGATTGC | species ID | (Minsavage et al., 1994) |
| RST33 | CACCATTCGTATCCCGGTG | species ID | (Minsavage et al., 1994) |
| XF1968-L | GGAGGTTTACCGAAGACAGAT | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| XF1968-R | ATCCACAGTAAAACCACATGC | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| XF2542-L | TTGATCGAGCTGATGATCG | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| XF2542-R | CAGTACAGCCTGCTGGAGTTA | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| ALM1 | CTGCAGAAATTGGAAACTTCAG | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| ALM2 | GCCACACGTGATCTATGAA | multiprimer PCR | (Hernandez-Martinez et al., 2006) |
| G1 | GAAGTCGTAACAAGG | ITS amplification | (Jensen et al., 1993) |
| L1 | CAAGGCATCCACCGT | ITS amplification | (Jensen et al., 1993) |
| XFPglA_Fw | GCCTCCGGTGCGACTGCTTC | $\begin{gathered} p g l A \\ \text { amplification }^{\mathrm{b}} \end{gathered}$ | This study |
| XFPglA_Rv | GCTGCGATTGGACACACATTG | $\begin{gathered} p g l A \\ \text { amplification } \end{gathered}$ | This study |
| ERIC1R | ATGTAAGCTCCTGGGGATTCAC | ERIC-PCR ${ }^{\text {c }}$ | (Versalovic et al., 1991) |
| ERIC2 | AAGTAAGTGATGGGGTGAGCG | ERIC-PCR | (Versalovic et al., 1991) |
| REP1R-I | IIIICGICGICATCIGGC | REP-PCR ${ }^{\text {d }}$ | (Versalovic et al., 1991) |
| REP2-I | ICGICTTATCIGGCCTAC | REP-PCR | (Versalovic et al., 1991) |

${ }^{2}$ Primers were synthesized by Bioneer, Inc (Alameda, CA).
${ }^{\mathrm{b}}$ Primers were designed within homologous regions surrounding the designated pglA gene in the complete genome sequences of $X$. fastidiosa strains 9 a 5 c , Temecula1, M12, and M23 available in NCBI's GenBank.
${ }^{c}$ ERIC-PCR: enterobacterial repetitive intergenic consensus PCR
${ }^{d}$ REP-PCR: repetitive extragenic palindromic PCR

ITS and pglA sequences were analyzed with Geneious Pro 5.4 (Biomatters LTD, Auckland, New Zealand) (Drummond et al., 2011). Alignment of sequences was performed with ClustalW2 (European Molecular Biology Laboratory's European Bioinformatics Institute, available at www.ebi.ac.uk) (Chenna et al., 2003). pglA sequences were translated into protein sequences using ExPASy Translate (Swiss Institute of Bioinformatics, http://expasy.org/) (Gasteiger et al., 2003).

Table 3. PCR and Electrophoresis Conditions for Tests Conducted in This Study
${ }^{\text {a }}$ PCR reagents and DNA template solutions were used at the following concentrations: GeneAmp® 10X PCR Buffer I containing 15 mM MgCl 2 (Applied Biosystems, Calsbad, CA); 10 X EasyA® reaction buffer (Agilent Technologies, Santa Clara, CA); EasyA® high fidelity PCR cloning enzyme ( $5 \mathrm{U} / \mu \mathrm{l}$ ) (Agilent Technologies, Santa Clara, CA); homemade Taq polymerase, $\sim 1.0 \mathrm{U} / \mu \mathrm{l}$; primers, $10 \mu \mathrm{M} ; \mathrm{MgCl}_{2}, 50 \mathrm{mM}$; dNTP mix, 10 mM ; DNA template, 1 $\mathrm{ng} / \mu$ l. Sterile $\mathrm{ddH}_{2} \mathrm{O}$ was used to bring the volume up to the desired reaction volume. Primer sequences are listed in Table 2.
${ }^{\mathrm{b}}$ PCR was performed in a DNA Engine Peltier Thermal Cycler (Bio-Rad, Hercules, CA).
${ }^{\text {c }}$ Agarose gels contained ethidium bromide for visualization of PCR products under UV light. PCR products were visualized and photographed with a KODAK Gel Logic 1500 Imaging System (Eastman Kodak Company, Rochester, New York). Unless specified, gels were run under standard conditions in 1X Tris-borate EDTA (TBE) buffer (Sambrook and Russell, 2001).

| Test | PCR reaction components ${ }^{\mathrm{a}}$ |  | PCR program ${ }^{\mathrm{b}}$ |
| :--- | :--- | :--- | :--- |$\quad$| Gel |
| :---: |
| electrophoresis ${ }^{\mathrm{c}}$ |

Rep-PCR Fingerprinting. Rep-PCRs were performed on the basis of two repetitive elements: enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and repetitive extragenic palindromic PCR (REP-PCR) (Versalovic et al., 1991). Primers used for ERIC-PCR and REP-PCR are listed in Table 2. The PCR cycling parameters and procedures and electrophoresis conditions for ERIC-PCR and REP-PCR are described in Table 3. Each rep-PCR was repeated at least twice. The presence or absence of bands between 100 bp and $1,650 \mathrm{bp}$ for ERIC-PCR and between 400 bp and 2,000 bp for REP-PCR was recorded. A consensus dataset for distinguishable bands that regularly appeared within these ranges in rep-PCR fingerprints was used for phylogenetic reconstruction.

Phylogenetic Analyses. DNA sequences of X. fastidiosa strains used for comparative analyses in this study were obtained from NCBI's GenBank (Appendix B).

ITS sequences from 49 strains of $X$. fastidiosa and one strain of Xanthomonas campestris pv. campestris were aligned with MUSCLE and cured in Gblocks (Méthodes et algorithmes pour la Bio-informatique LIRMM, http://www.phylogeny.fr/version2_cgi/index.cgi) (Dereeper et al., 2008). A maximum likelihood (ML) search for the best-scoring ML tree of the cured ITS sequences was performed in RAxML using the rapid bootstrapping algorithm and the GTRMIX model of evolution (Appendix C) (Stamatakis, 2006; Stamatakis et al., 2008). Analyses were run with 1000 bootstrap replicates. A Markov Chain Monte Carlo (MCMC) search for the cured ITS sequences was performed in MrBayes 3.1 (Appendix D) (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Ronquist and Huelsenbeck, 2003). Four runs each with four chains were tested until the standard deviation of the split frequencies reached 0.01 . A starting tree obtained from the ML analysis was used for Bayesian analysis.

The aligned $p g l A$ and $p g l A$-homologous sequences from 13 strains of $X$. fastidiosa were analyzed in RAxML and MrBayes, as previously described, except that MCMC search runs were tested until the standard deviation of the split frequencies reached 0.02 and no starting tree was used (Appendices C and D).

Phylogenetic reconstruction using the unweighted pair group method with arithmetic mean (UPGMA) was performed in MEGA5 for the individual and combined datasets of binary data for ERIC-PCR and REP-PCR (Tamura et al., 2011). Analyses were run with 1000 bootstrap replicates. A MCMC search for the individual and combined datasets for ERIC-PCR and REPPCR was performed in MrBayes 3.1 (Appendix D). Four runs each with four chains were tested until the standard deviation of the split frequencies reached 0.01 .

A MCMC search for the total combined data set of 49 strains of $X$. fastidiosa and one strain of $X$. campestris pv. campestris including the aligned and cured ITS sequences, the aligned pglA sequences, and the binary data from ERIC-PCR and REP-PCR was performed in MrBayes 3.1 (Appendix D). Four runs each with four chains were tested until the standard deviation of the split frequencies reached 0.02 . A starting tree obtained from the ML analysis for the aligned and cured ITS sequences was used for the MCMC search. Since $p g l A$ sequences were only available for strains whose complete genomes have been published, these sequences were included with the ITS sequences in the combined dataset so that a pglA sequence from a particular subspecies and host was included with the ITS sequence of a strain from the same subspecies and host. These sequences were included as follows: 9a5c pglA sequence included with strain CI.X0; Temeculal pglA sequence included with strain PD 95-2; XF GB514 pglA sequence included with strain PD 95-4; M12 pglA sequence included with strain Dixon; and M23 pglA sequence included with strain ALS1.

Convergence of all Bayesian analyses was tested using the cumulative and compare functions in AWTY (http://king2.scs.fsu.edu/CEBProjects/awty/awty start.php) (Nylander et al., 2008). Phylogenetic trees were obtained from RAxML for ITS sequence, pglA sequence, and total combined analyses and from MEGA5 for ERIC-PCR and REP-PCR data analyses. Bootstrap values from ML analyses or UPGMA analyses greater than $70 \%$ and posterior probabilities from MCMC analyses greater than 0.95 were included on phylogenetic trees. $X$. fastidiosa strain XF A-10 was not included in the ML searches or in the MCMC analysis of the total combined dataset due to a lack of sequence data.

## RESULTS

Genetic Verification of $X$. fastidiosa Strains Isolated from Pecan and Other Plant Hosts in Louisiana. A DNA band approximately 733 bp in size, the expected product size for $X$. fastidiosa when using X. fastidiosa-specific primers RST31 and RST33, was produced from all bacterial strains isolated from pecan and other hosts (grapevine, oleander, and sycamore) from Louisiana and used in this study (Fig. 1; data not shown for XF A-10 and XF B-10).

Multiprimer PCR Assay of $\boldsymbol{X}$. fastidiosa from Louisiana. Three DNA bands approximately $412 \mathrm{bp}, 521 \mathrm{bp}$, and 638 bp in size, the expected sizes of the products amplified from the multiprimer PCR assay for the $X$. fastidiosa subsp. multiplex Almond Leaf Scorch (ALS) II genotype, were amplified from each of the pecan and sycamore strains from Louisiana (Fig. 2; data not shown for XF A-10 and XF B-10) (Hernandez-Martinez et al., 2006). A single band approximately 412 bp in size, the expected size of the product amplified from the multiprimer PCR assay for the $X$. fastidiosa subsp. fastidiosa Pierce's disease (PD) genotype, was amplified from the Louisiana grapevine strain, XF A-08 (Fig. 2) (Hernandez-Martinez et al., 2006). A single band approximately 638 bp in size, the expected size of the product amplified

Figure 1. Agarose gel showing the expected 733-bp PCR product using primers RST31 and RST33, specific for Xylella fastidiosa, for bacterial strains isolated from diseased grapevine, oleander, pecan, and sycamore from Louisiana. A strain of Burkholderia glumae and sterile distilled deionized water (ddH2O) were used as negative controls. Samples were arranged as follows: lane 1: 1 kb plus ladder (Invitrogen); lane 2: XF A-05 (pecan); lane 3: XF B-05 (pecan); lane 4: XF C-05 (pecan); lane 5: XF A-06 (pecan); lane 6: XF D-07 (pecan); lane 7: XF A-08 (grapevine); lane 8: XF Oleander (oleander); lane 9: XF A-07 (sycamore); lane 10: B. glumae 336gr-1; and lane 11: sterile ddH2O. Strains XF A-10 and XF B-10 from pecan (not pictured) also produced the expected PCR products from primers RST31 and RST33.

Figure 2. Agarose gel (1.5\%) showing multiprimer PCR products of strains of Xylella fastidiosa isolated from diseased grapevine, oleander, pecan, and sycamore from Louisiana. A strain of Burkholderia glumae and sterile distilled deionized water $\left(\mathrm{ddH}_{2} \mathrm{O}\right)$ were used as negative controls. Samples were arranged as follows: lane 1: 1 kb plus ladder (Invitrogen); lane 2: XF A05 (pecan); lane 3: XF B-05 (pecan); lane 4 XF C-05 (pecan); lane 5: XF A-06 (pecan); lane 6: XF D-07 (pecan); lane 7: XF A-08 (grapevine); lane 8: XF Oleander (oleander); lane 9: XF A-07 (sycamore); lane 10: B. glumae 336gr-1; lane 11: sterile $\mathrm{ddH}_{2} \mathrm{O}$; and lane 12: 1 kb plus ladder. Strains XF A-10 and XF B-10 from pecan (not pictured) produced all three possible PCR products from the multiprimer PCR.
from the multiprimer PCR assay for the $X$. fastidiosa subsp. sandyi Oleander Leaf Scorch (OLS) genotype, was amplified from the Louisiana oleander strain, XF Oleander (Fig. 2) (HernandezMartinez et al., 2006).

## Sequence Analyses of the 16S-23S rDNA ITS Region of $X$. fastidiosa Strains from

Louisiana. ITS sequences were obtained from nine strains of $X$. fastidiosa used in this study (Appendix E). ClustalW2 alignment of the ITS sequences trimmed to the same starting and ending points (Appendix F) showed that the ITS sequences from all strains of $X$. fastidiosa from pecan and sycamore from Louisiana were identical. These strains had a sequence identity of $98 \%$ when compared to the grapevine (XF A-08) and oleander (XF Oleander) strains from Louisiana (Appendix F). A basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) search of the pecan and sycamore ITS sequence against the complete genomes of $X$. fastidiosa showed the highest sequence identity to $X$. fastidiosa subsp. multiplex strain M12 from almond (100\%), followed by $X$. fastidiosa subsp. pauca strain 9a5c from citrus (99\%), and $X$. fastidiosa subsp. fastidiosa strains Temeculal and GB514 from grapevine and M23 from almond (98\%) (Appendix G) (Altschul et al., 1990). A BLAST search of the ITS sequence from grapevine strain XF A-08 showed the highest sequence identity (100\%) to $X$. fastidiosa subsp. fastidiosa strains Temecula1 and GB514 from grapevine and M23 from almond (Appendix G). A BLAST search of the ITS sequence from oleander strain XF Oleander showed the highest sequence identity (99\%) to $X$. fastidiosa subsp. fastidiosa strains Temeculal and GB514 from grapevine and M23 from almond (Appendix G).

ITS sequences obtained for strains of $X$. fastidiosa in this study were deposited in GenBank under accession numbers JN092378 through JN092385 (Appendix B).

Sequence Analyses of pglA of $\boldsymbol{X}$. fastidiosa Strains from Louisiana. DNA sequences of pglA encoding a polygalacturonase, a major virulence factor of $X$. fastidiosa (Roper et al., 2007), were obtained from eight strains of $X$. fastidiosa used in this study (Appendix H). ClustalW2 alignment of the pglA sequences (Appendix I) showed that the pglA sequences from all $X$. fastidiosa strains from pecan and sycamore from Louisiana were identical. These strains had a sequence identity of $98 \%$ when compared to the grapevine (XF A-08) and oleander (XF Oleander) strains from Louisiana (Appendix I). A BLAST search of the pecan and sycamore pglA sequence against the complete genome sequences of $X$. fastidiosa showed the highest sequence identity (99\%) to X. fastidiosa subsp. multiplex strain M12 from almond, followed by X. fastidiosa subsp. fastidiosa strains Temecula1 and GB514 from grapevine and M23 from almond (98\%), and X. fastidiosa subsp. pauca strain 9a5c from citrus (97\%) (Appendix J). A BLAST search of the pglA sequence from grapevine strain XF A-08 showed the highest sequence identity (100\%) to $X$. fastidiosa subsp. fastidiosa strains Temecula1 and GB514 from grapevine and M23 from almond (Appendix J). A BLAST search of the pglA sequence from oleander strain XF Oleander showed the highest sequence identity (98\%) to X. fastidiosa subsp. fastidiosa strains Temecula1 and GB514 from grapevine and M23 from almond and to $X$. fastidiosa subsp. multiplex strain M12 from almond (Appendix J).

Early termination, which is present in the pglA gene of strain 9a5c from citrus (Van Sluys et al., 2003), was not found in any of the pglA sequences from strains of $X$. fastidiosa from Louisiana (Appendix K).
pglA sequences obtained from strains of $X$. fastidiosa in this study were deposited in GenBank under accession numbers JN092387 through JN092394 (Appendix B).

Rep-PCR Analyses of $\boldsymbol{X}$. fastidiosa Strains from Louisiana. Bands in ten band classes between 100 bp and 1,650 bp for ERIC-PCR and in nine band classes between 400 bp and 2,000 bp for REP-PCR were scored as being present or absent for each X. fastidiosa strain tested (Appendix L).

All pecan strains, regardless of the cultivar from which they were isolated, and the sycamore strain produced the same ERIC-PCR fingerprint (Fig. 3a). Obvious band differences were present in the ERIC-PCR fingerprints between the grapevine and oleander strains and between these strains and those from pecan and sycamore (Fig. 3a). Namely, bands approximately $155 \mathrm{bp}, 550 \mathrm{bp}$, and 880 bp in size were present only in grapevine strain XF A-08 and oleander strain XF Oleander but not in any of the other strains; bands approximately 160 bp and 950 bp in size were present in all pecan strains and in sycamore strain XF A-07 but not in grapevine strain XF A-08 or oleander strain XF Oleander; bands approximately 300 bp and 420 bp in size were present in all strains except oleander strain XF Oleander; and a band approximately $1,275 \mathrm{bp}$ in size was present in oleander strain XF Oleander but not in any of the other strains (Fig. 3a)

REP-PCR fingerprints of pecan strains, however, showed variation among some pecan strains (Fig. 4a). Pecan cv. Cape Fear strain XF A-06 and sycamore strain XF A-07 produced a band approximately 900 bp in size that was not present in any of the other strains tested (Fig. 4a). Pecan cv. Oconee strain XF A-10 failed to produce a band approximately $1,900 \mathrm{bp}$ in size that was present in all of the other pecan strains and in sycamore strain XF A-07, but produced a slightly larger band, approximately $1,950 \mathrm{bp}$ in size, that was not present in any of the other strains tested.

Figure 3. (a) Agarose gel (1.8\%) showing ERIC-PCR product fingerprints from 10 strains of Xylella fastidiosa isolated from diseased grapevine, oleander, pecan, and sycamore from Louisiana. Sterile distilled deionized water $\left(\mathrm{ddH}_{2} \mathrm{O}\right)$ was used as a negative control. Samples were arranged as follows: lane 1: 1 kb plus ladder (Invitrogen); lane 2: XF A-05 (pecan - CF); lane 3: XF B-05 (pecan - CF); lane 4: XF C-05 (pecan - CF); lane 5: XF A-06 (pecan - CF); lane 6: XF D-07 (pecan - CF); lane 7: XF A-10 (pecan - O); lane 8: XF B-10 (pecan - D); lane 9: XF A-08 (grapevine); lane 10: XF Oleander (oleander); lane 11: XF A-07 (sycamore); lane 12: Burkholderia glumae 336gr-1; lane 13: sterile $\mathrm{ddH}_{2} \mathrm{O}$; and lane 14: 1 kb Plus ladder. Pecan cultivar designations: $\mathrm{CF}=$ Cape Fear; $\mathrm{D}=$ Desirable; $\mathrm{O}=$ Oconee. (b) Phylogram constructed from the ERIC-PCR fingerprints shown in (a). Branch tip labels include the plant host and cultivar, when applicable, from which the strain of $X$. fastidiosa was obtained and the strain name (parentheses). Phylogram was obtained from a UPGMA analysis (1000 bootstrap replicates) using MEGA5. Bootstrap values ( $>70 \%$ ) are shown above branch nodes; posterior probabilities $(>0.95)$ obtained from Bayesian inference in MrBayes 3.1 are shown below branch nodes.
(Figure 3 continued)

Figure 4. (a) Agarose gel (1.8\%) showing REP-PCR product fingerprints from 10 strains of Xylella fastidiosa isolated from diseased grapevine, oleander, pecan, and sycamore from Louisiana. Sterile distilled deionized water $\left(\mathrm{ddH}_{2} \mathrm{O}\right)$ was used as a negative control. Samples were arranged as follows: lane 1: 1 kb plus ladder (Invitrogen); lane 2: XF A-05 (pecan - CF); lane 3: XF B-05 (pecan - CF); lane 4: XF C-05 (pecan -.CF); lane 5: XF A-06 (pecan - CF); lane 6: XF D-07 (pecan - CF); lane 7: XF A-10 (pecan - O); lane 8: XF B-10 (pecan - D); lane 9: XF A-08 (grapevine); lane 10: XF Oleander (oleander); lane 11: XF A-07 (sycamore); lane 12: Burkholderia glumae 336gr-1; lane 13: sterile $\mathrm{ddH}_{2} \mathrm{O}$; and lane 14: 1 kb plus ladder. Pecan cultivar designations: $\mathrm{CF}=$ Cape Fear; $\mathrm{D}=$ Desirable; $\mathrm{O}=$ Oconee. (b) Phylogram constructed from the REP-PCR fingerprints shown in (a). Branch tip labels include the plant host and cultivar, when applicable, from which the strain of $X$. fastidiosa was obtained and the strain name (parentheses). Phylogram was obtained from a UPGMA analysis (1000 bootstrap replicates) using MEGA5. Bootstrap values ( $>70 \%$ ) are shown above branch nodes; posterior probabilities $(>0.95)$ obtained from Bayesian inference in MrBayes 3.1 are shown below branch nodes.
(Figure 4 continued)

Phylogenetic Analyses of $\boldsymbol{X}$. fastidiosa Strains from Louisiana. Three main groups were recovered from Bayesian inference and UPGMA using the ERIC-PCR dataset (Fig. 3b). Group I, which was well supported by Bayesian posterior probability (0.98) and UPGMA bootstrap (99\%), was monophyletic and included all strains of $X$. fastidiosa from pecan and sycamore (Fig. 3b). Group II included only the grapevine strain and was separate from all other strains of $X$. fastidiosa from Louisiana. Group III included only the oleander strain and was also separate from all other strains of $X$. fastidiosa from Louisiana.

Three main groups were recovered from Bayesian inference and UPGMA using the REPPCR dataset (Fig. 4b). Group I, which was well supported by Bayesian posterior probability (0.97) and UPGMA bootstrap (99\%), was monophyletic and included all strains of $X$. fastidiosa from pecan and sycamore (Fig. 4b). Three subgroups, however, were present within Group I: one subgroup included strain XF A-06 from the pecan cultivar Cape Fear and strain XF A-07 from sycamore (UPGMA bootstrap $=90 \%$ ); the second subgroup included only strain XF A-10 from the pecan cultivar Oconee; and the third subgroup included four strains from the cultivar Cape Fear (XF A-05, XF B-05, XF C-05, and XF D-07) and strain XF B-10 from the pecan cultivar Desirable (UPGMA bootstrap $=78 \%$ ) (Fig. 4b). Group II and Group III included only the grapevine strain and the oleander strain, respectively, separating each of them from all other strains.

A UPGMA phylogram recovered from the combined ERIC-PCR and REP-PCR datasets was similar to the REP-PCR UPGMA phylogram with similar support from bootstrap values and posterior probabilities except that it showed the pecan-sycamore subgroup to be more closely related to the larger pecan subgroup (Fig. 5).

Figure 5. Phylogram constructed from the combined ERIC-PCR and REP-PCR product fingerprints. Branch tip descriptions include the plant host and cultivar, when applicable, from which the strain of X. fastidiosa was obtained and the strain name (parentheses). Phylogram was obtained from a UPGMA analysis (1000 bootstrap replicates) using MEGA5. Bootstrap values $(>70 \%)$ are shown above branch nodes; posterior probabilities ( $>0.95$ ) obtained from Bayesian analysis in MrBayes 3.1 are shown below branch nodes.

The ML phylogram of aligned and cured 16S-23S ITS regions from 49 strains of $X$. fastidiosa and from one strain of $X$. campestris pv . campestris included as an outgroup placed the majority of the $X$. fastidiosa strains into six major monophyletic or paraphyletic groups with bootstrap values greater than $84 \%$ (Fig. 6). The majority of these groups were also strongly supported by posterior probabilities greater than 0.95 . Group I was monophyletic and included strains from citrus and coffee. Group II was paraphyletic with unresolved relationships among strains from a variety of hosts, including the pecan and sycamore strains from Louisiana and strains from almond and peach. Group III was a monophyletic group within Group II, and included strains of $X$. fastidiosa from chitalpa (Randall et al., 2009). Group IV was a

Figure 6. Phylogram of the 16S-23S rDNA intergenic transcribed spacer (ITS) sequence dataset from 49 strains of Xylella fastidiosa and one strain of Xanthomonas campestris pv. campestris included as an outgroup. Branch tip labels include the host and strain name (parentheses) of $X$. fastidiosa. ITS sequences obtained from this study are indicated in bold. Sequences from all other strains were obtained from NCBI's GenBank (accession numbers in Appendix B). Phylogram was obtained from a maximum likelihood (ML) search using RAxML. Bootstrap values ( $>70 \%$ ) from 1000 bootstrap replicates from the ML search are shown above branch nodes; posterior probabilities ( $>0.95$ ) obtained from Bayesian inference in MrBayes 3.1 are shown below branch nodes.
monophyletic group also recovered within Group II that included strains of $X$. fastidiosa from oak. Group V was monophyletic and included strains of $X$. fastidiosa from oleander, including the Louisiana oleander strain. Group VI was monophyletic and included strains of $X$. fastidiosa from almond and grapevine, including the Louisiana grapevine strain.

The ML phylogram of the aligned pglA sequences from 13 strains of $X$. fastidiosa, including citrus strain 9 a 5 c used as an outgroup, separated the strains into four groups (Fig. 7). The pecan and sycamore strains formed a monophyletic group with $X$. fastidiosa subsp. multiplex strain M12 from almond. The Louisiana grapevine strain formed a monophyletic group with strains of $X$. fastidiosa subsp. fastidiosa from grapevine and almond. The Louisiana oleander strain did not group with other strains of X. fastidiosa. X. fastidiosa subsp. pauca strain 9a5c from citrus also did not group with other strains of $X$. fastidiosa. Most of the major groups were strongly supported with bootstrap values greater than $85 \%$ and posterior probabilities of 0.99 (Fig. 7).

The MCMC phylogram of the total combined dataset of 49 strains of $X$. fastidiosa and one strain of $X$. campestris pv. campestris including the aligned and cured ITS sequences, the aligned pglA sequences, and the binary data from ERIC-PCR and REP-PCR placed the majority of the $X$. fastidiosa strains into six major monophyletic or paraphyletic groups with bootstrap values greater than $84 \%$ (Fig. 8). Though the location of groups on the phylogram differed in comparison to the phylogram recovered from the cured ITS sequences (Fig. 6), the different monophyletic and paraphyletic groups in this analysis were comprised of the same strains of $X$. fastidiosa as the previous analysis. The pecan and sycamore strains were still part of a large paraphyletic group that included other strains of $X$. fastidiosa from almond and peach. This analysis showed increased posterior probability support for a number of groups (Fig. 8).

Figure 7. Phylogram of pglA sequences from 13 strains of Xylella fastidiosa with the citrus strain 9 a 5 c used as an outgroup. Branch tip labels include the host strain name (parentheses) of $X$. fastidiosa; the known subspecies of $X$. fastidiosa are indicated below each branch tip label. $p g l A$ sequences obtained from this study are indicated in bold. Sequences from all other strains were obtained from NCBI's GenBank. Phylogram was obtained from a maximum likelihood (ML) search using RAxML. Bootstrap values ( $>70 \%$ ) from 1000 bootstrap replicates from the ML search in RAxML are shown above branch nodes; posterior probabilities ( $>0.95$ ) obtained from Bayesian inference in MrBayes 3.1 are shown below branch nodes.

Figure 8. Phylogram of the combined dataset constructed from available 16S-23S rDNA intergenic transcribed spacer (ITS) sequences, pglA sequences, and ERIC-PCR and REP-PCR product fingerprints from 49 strains of Xylella fastidiosa and one strain of Xanthomonas campestris pv. campestris included as an outgroup. Pecan strain XF A-10 was not included in the analysis due to a lack of sequence data. Since $p g l A$ sequences were only available for strains whose complete genomes have been published, these sequences were included with the ITS sequences in the combined dataset as follows: 9a5c pglA sequence included with strain CI.X0; Temeculal pglA sequence included with strain PD 95-2; XF GB514 pglA sequence included with strain PD 95-4; M12 pglA sequence included with strain Dixon; and M23 pglA sequence included with strain ALS1. Branch tip labels include the plant host from which the strain of $X$. fastidiosa was obtained and the strain name (parentheses). ITS sequences, ERIC-PCR and REPPCR data obtained from this study are indicated in bold. Sequences from all other strains were obtained from GenBank (accession numbers in Appendix B). Phylogram was obtained from Bayesian analysis in MrBayes 3.1. Bootstrap values ( $>70 \%$ ) from 1000 bootstrap replicates obtained from the maximum likelihood search in RAxML of the 16S-23S ITS sequences cured in Gblocks and shown in Fig. 6 are shown above branch nodes; posterior probabilities ( $>0.95$ ) obtained from the Bayesian inference of the combined data set in MrBayes 3.1 are shown below branch nodes.

Convergence of all Bayesian analyses was verified using the cumulative and compare functions in AWTY (Appendix M).

## DISCUSSION

X. fastidiosa is a complex plant pathogen that infects and causes disease in a wide range of hosts (Purcell and Hopkins, 1996). Subspecies classifications have been based largely on molecular techniques and host plant infectivity. However, genetic differences within subspecies and even within strains from the same host have also been observed (Almeida et al., 2008; Mehta et al., 2001; Scally et al., 2005). Recombination has also been shown to occur between subspecies and between strains from different hosts (Almeida et al., 2008; Scally et al., 2005; Schuenzel et al., 2005). The presence of multiple subspecies and strains of $X$. fastidiosa in the same geographic region with common insect vectors may increase the opportunity for continuing diversification within this species and the possibility of the occurrence of new diseases in hosts that have yet to be infected by $X$. fastidiosa (Nunney et al., 2010).

Pecan, the only native nut grown commercially in the United States (Perez and Pollack, 2003), is produced in 15 states (Agricultural Statistics 2010, USDA-NASS), including California, New Mexico, Texas, Louisiana, Georgia, and Florida, where diseases caused by $X$. fastidiosa have been reported and are known to occur. Despite this and the fact that $X$. fastidiosa causes disease in pecan, pecan strains of $X$. fastidiosa have rarely been studied. Consequently, genetic and taxonomic information regarding pecan strains of $X$. fastidiosa is very limited. In this investigation, various molecular techniques and phylogenetic methods were used to study the taxonomy of $X$. fastidiosa strains isolated from pecan and other hosts in Louisiana at a subspecific level and to determine the $X$. fastidiosa subspecies grouping of the strains from pecan. Three strains of $X$. fastidiosa isolated from grapevine, oleander, and sycamore growing in

Louisiana were included for comparative analysis in experiments as strains from these host plants typically identify as $X$. fastidiosa subsp. fastidiosa, $X$. fastidiosa subsp. sandyi, and $X$. fastidiosa subsp. multiplex, respectively. To the best of our knowledge, this is the first phylogenetic study of $X$. fastidiosa strains from pecan and from Louisiana.

The multiprimer PCR assay identified strains of $X$. fastidiosa from Louisiana by genotype and subspecies (Fig. 2). The amplification of three products from the multiprimer PCR assay indicated that strains of $X$. fastidiosa from pecan and sycamore from Louisiana are of the ALSII genotype that belongs to $X$. fastidiosa subsp. multiplex and includes strains that caused mild PD symptoms in grapevine (Fig. 2) (Almeida and Purcell, 2003a; Hernandez-Martinez et al., 2006). A limitation of the multiprimer PCR assay is that it was designed using strains of $X$. fastidiosa from a limited number of hosts, specifically almond, grapevine, and oleander. It is uncertain if strains from different hosts will produce the same results. Host-plant inoculations that should determine the relevance of the pecan strains as an ALSII genotype and their ability to cause disease symptoms in grapevine are currently being conducted. The multiprimer PCR assay identified the grapevine strain from Louisiana as the PD genotype of $X$. fastidiosa subsp. fastidiosa and the oleander strain from Louisiana as the OLS genotype of X. fastidiosa subsp. sandyi (Fig. 2).

Sequence analysis of the 16S-23S rDNA ITS region, which has been used in previous studies to identify strains of $X$. fastidiosa below the species level, indicated that the pecan and sycamore ITS sequences were identical and most similar to other $X$. fastidiosa subsp. multiplex strains (Jensen et al., 1993; Martinati et al., 2005; Mehta and Rosato, 2001). Our findings that the ITS sequences of the pecan strains from Louisiana were identical to each other and that the ITS sequence of the grapevine strain from Louisiana was identical to those of the completely
sequenced grapevine strains were not surprising since a previous study showed that, with the exception of almond strains, all strains from the same host had the same ITS sequence (Hendson et al., 2001). Phylogenetic analysis of the ITS region supported the subspecies groupings determined from the multiprimer PCR assay (Figs. 2 and 6). In addition, the major groups of $X$. fastidiosa strains identified in this study were consistent with groups from previous phylogenetic analyses based on ITS sequences, with the exception that the strains from chitalpa formed a monophyletic group within the unresolved subspecies multiplex group (Fig. 6) (Hendson et al., 2001; Martinati et al., 2005; Mehta and Rosato, 2001).

A frameshift causing early termination in the polygalacturonase precursor gene, pglA, was found in citrus and coffee strains but not in almond, grapevine, mulberry, or oleander strains of X. fastidiosa (Van Sluys et al., 2003). A frameshift was not found in this study in pglA of pecan, grapevine, oleander, or sycamore strains from Louisiana. It was suggested that this frameshift possibly made $p g l A$ nonfunctional and accounted for the less aggressive nature of citrus variegated chlorosis strains compared to Pierce's disease strains of X. fastidiosa (Almeida et al., 2001; Van Sluys et al., 2003). Polygalacturonase is required for colonization and pathogenicity in grapevine but is not necessary for the development of disease in citrus (Roper et al., 2007; Van Sluys et al., 2003). Despite the absence of a frameshift in pglA of X. fastidiosa pecan, oleander and sycamore strains, it is unknown if this gene is required for colonization and pathogenicity of these strains. As with the ITS sequences, all $p g l A$ sequences from pecan and sycamore were identical. These sequences were more closely related to the $X$. fastidiosa subsp. multiplex strain than to any other subspecies. The pglA sequence from grapevine in Louisiana was not identical to other grapevine or subsp. fastidiosa strains, but was more closely related to these strains than to other strains. Sequence analysis of $p g l A$ separated strains of $X$. fastidiosa
into the same subspecies groups as the multiprimer PCR assay, providing further support for these groups (Figs. 2 and 7).

ERIC-PCR and REP-PCR separated the Louisiana pecan and sycamore strains from the Louisiana grapevine strain and the Louisiana oleander strain (Figs. 3 and 4). REP-PCR revealed genetic differences within the strains of $X$. fastidiosa from pecan while ERIC-PCR failed to detect any differences (Figs. 3 and 4). Similar results were reported for X. fastidiosa strains from citrus in which REP-PCR banding patterns were more discriminative than the ERIC-PCR banding patterns (Mehta et al., 2001). While the differences in the citrus strain REP-PCR profiles corresponded to differences in geographic location (Mehta et al., 2001), differences in the strains of $X$. fastidiosa from pecan were observed within the same geographic location and the same cultivar in this study (Table 1, Fig. 4). In the REP-PCR, one of the five pecan strains from the cultivar Cape Fear, XF A-06, clustered with and was more closely related to the sycamore strain, XF A-07, than to the other pecan strains (Fig. 4). This subgroup was more related to the pecan strain from the cultivar Oconee, XF A-10, than to the subgroup containing four other pecan strains from the cultivar Cape Fear (XF A-05, XF B-05, XF C-05, and XF D07) and the pecan strain from the cultivar Desirable, XF B-10 (Fig. 4). The significance of the genetic variation observed among pecan strains in the REP-PCR is not currently known.

The phylogenetic analysis of the combined dataset of the ITS sequences, pglA sequences, and ERIC-PCR and REP-PCR data strengthened support for a number of groups identified in this study but did not place the pecan strains in a separate monophyletic group within the $X$. fastidiosa subsp. multiplex group (Fig. 8). Additional strains from pecan and other hosts and additional genetic information may be necessary to distinguish the pecan strains as a separate monophyletic group. A multilocus sequence typing (MLST) analysis, which identified different
clonal complexes within X. fastidiosa subsp. multiplex (Scally et al., 2005), may be able to identify a new clonal complex for pecan strains or may group pecan strains with other similar strains, possibly sycamore strains, based on this study. Amplification and sequencing of part of these genes using primers designed for the original MLST system analysis for X. fastidiosa were attempted (Appendix N ); however, because returned sequences were not always readable, this sequence data was not included in phylogenetic analyses (Appendix O). Further attempts to obtain sequences with the newly designed MLST primers (Yuan et al., 2010) should be pursued.

Results from the various experiments conducted in this study consistently grouped the strains of $X$. fastidiosa from pecan together with each other and with the strain of $X$. fastidiosa from sycamore from Louisiana and as part of a larger group that included strains of $X$. fastidiosa subsp. multiplex from other host plants. For this reason, strains of $X$. fastidiosa from pecan should be considered a part of $X$. fastidiosa subsp. multiplex. In addition, the analyses conducted in this study placed the Louisiana grapevine strain with other grapevine strains from X. fastidiosa subsp. fastidiosa and the Louisiana oleander strain with other oleander strains from $X$. fastidiosa subsp. sandyi.

Studies on PBLS incidence in the research orchards at the Pecan Research-Extension Station have shown consistent disease spread within some cultivars (Sanderlin et al., 2009). Disease severity and rate of spread also differ between pecan cultivars. Even though the majority of the pecan strains utilized in this study came from a single location and from a single cultivar, genetic variation was detected within these strains. The genetic differences observed between strains of $X$. fastidiosa from pecan from the same location and from the same cultivar in the REP-PCR test suggest that these strains may not be clones originating from a single source of infection. However, it cannot be ruled out that some mutation occurred to cause the genetic
variation seen in the REP-PCR while a single strain spread throughout the orchards. To determine the biological meaning of these observed genetic variations within the pecan strains of X. fastidiosa, additional studies including host specificity tests with different plant hosts and pecan cultivars are needed.

One of the difficulties in working with $X$. fastidiosa is that it is very fastidious and slow growing. Isolation attempts from pecan over several years have had low success rates and yielded few strains. Conscious efforts should be made to build a collection of $X$. fastidiosa pecan strains suitable for future studies. This collection should include strains from various cultivars as well as from various geographic locations.

This work provides a foundation for studies with additional strains of $X$. fastidiosa from Louisiana to determine variation that exists in this wide-spread pathogen within Louisiana and other southeastern U.S. states. Future studies that examine new diseases and the systematics of X. fastidiosa, and that continue to investigate the diversification of the species, will help to provide a better understanding of the population dynamics of this pathogen and to highlight research focus areas for effective management of plant diseases caused by this pathogen.

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CHAPTER 3: CONCLUSIONS
X. fastidiosa threatens the pecan industry by causing reduced nut yields in infected trees. Currently, management strategies are limited to reducing or eliminating the introduction of this pathogen into pecan orchards. No resistant cultivars have been identified. Furthermore, there is no treatment or cure once trees become infected, and disease tends to occur annually. This situation amounts to decreased income for growers with infected trees in their production orchards. Many studies are being conducted in other hosts infected by X. fastidiosa, and several subspecies with some apparent host specificity have been identified. A considerable amount of information about pathogen transmission has been determined for $X$. fastidiosa in pecan, but there is little information, particularly at the molecular level, on strains infecting pecan. Thus far, pecan pathogenic strains of $X$. fastidiosa have not been grouped to subspecies. This information is needed to develop more effective pecan bacterial leaf scorch (PBLS) management strategies, particularly to make it possible to clearly identify alternative hosts that could serve as reservoirs of inoculum for $X$. fastidiosa diseases in Louisiana.

Multiprimer PCR and phylogenetic analyses using nucleotide sequence data from the $16 \mathrm{~S}-23 \mathrm{~S}$ rDNA ITS region and pglA consistently identified strains of $X$. fastidiosa from pecan as X. fastidiosa subsp. multiplex. ERIC-PCR and REP-PCR analyses supported phylogenetic analyses. Based on these data, strains of $X$. fastidiosa from pecan should be considered a part of X. fastidiosa subsp. multiplex. Results from these experiments also identified the Louisiana grapevine strain as $X$. fastidiosa subsp. fastidiosa, the Louisiana oleander strain as $X$. fastidiosa subsp. sandyi, and the Louisiana sycamore strain as X. fastidiosa subsp. multiplex. In addition, REP-PCR showed that genetic variation exists among strains of $X$. fastidiosa from pecan. This genetic variation has not yet been explored, but warrants further study, particularly since the same genetic variant was observed in the strain of $X$. fastidiosa from sycamore.

Despite the presence of multiple subspecies of $X$. fastidiosa present in Louisiana, this investigation only identified $X$. fastidiosa subsp. multiplex in pecan. It is possible that pecan can only be infected by $X$. fastidiosa susbp. multiplex strains or by particular strains of $X$. fastidiosa subsp. multiplex. Host range inoculation tests with strains of $X$. fastidiosa from other subspecies and from other hosts within $X$. fastidiosa subsp. multiplex should be conducted to determine infectivity of pecan by different subspecies and strains. The knowledge from these tests would further aid in PBLS management methods by determining which plant species may harbor pecan-infecting strains of $X$. fastidiosa and how these species should be managed when adjacent to pecan orchards. As of now, a possible threat only exists when host plants infected with $X$. fastidiosa subsp. multiplex are growing in and around pecan orchards. Even if plant hosts do not harbor strains of $X$. fastidiosa known to infect pecan, genetic recombination, which has been reported within and between subspecies, could yield novel strains able to infect pecan. Further studies on this pathogenic bacterial species, on its mechanisms of pathogenicity, and on the factors that determine host specificity will provide additional information that may aid in the understanding of this species as a whole.

## APPENDIX A: SUBMISSION TO PLANT DISEASE

A manuscript containing a major portion of Chapter 2, the thesis abstract, and Appendix B, coauthored by R. S. Sanderlin, A. R. McTaggart, and J. H. Ham, has been submitted for publication to Plant Disease under the title, "A systematic study of the 16S-23S rRNA internal transcribed spacer region, pglA, and ERIC-PCR and REP-PCR fingerprints reveals that Xylella fastidiosa strains from pecan are part of $X$. fastidiosa subsp. multiplex".

| Manuscript ID | Manuscript Title | Date Created | Date Submitted | Status |
| :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & \text { PDIS-09-11- } \\ & 0730-R E \end{aligned}$ | A systematic study of the $16 \mathrm{~S}-$ 23S rRNA internal transcribed spacer region, pglA, and ERICPCR and REP-PCR fingerprints reveals that Xylella fastidiosa strains from pecan are part of $X$. fastidiosa subsp. multiplex | $\begin{aligned} & \text { 01-Sep- } \\ & 2011 \end{aligned}$ | $\begin{aligned} & \text { 02-Sep- } \\ & 2011 \end{aligned}$ | SE: Charkowski, Amy EIC: Davis, R. Michael NE: Not Assigned ADM: Gold, Linda PROD: Not Assigned <br> Awaiting SE Decision |

Email correspondence between Dr. Jong Hyun Ham, advisor, and Dr. Amy Charkowski, Plant Disease senior editor assigned to my manuscript:

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jham@agcenter.lsu.edu wrote:
20-Sep-2011
PDIS-09-11-0730-RE - A systematic study of the 16S-23S rRNA internal
transcribed spacer region, pglA, and ERIC-PCR and REP-PCR fingerprints
reveals that Xylella fastidiosa strains from pecan are part of X.
fastidiosa subsp. multiplex
Dear Amy:
My student, Rebecca Melanson, plans to defend her master's thesis on
November. I want to ask your permission to include the submitted manuscript
(PDIS-09-11-0730-RE) into her master's thesis as a chapter. Submission status
of the manuscript will be indicated on the front page of the chapter and the
manuscript will be edited appropriately for the thesis.
By the way, would you also let me know when reviewers will be assigned for
our manuscript?
Thank you for processing our manuscript and I will look forward to hearing
from you soon.
Best regards,
```

Jong
From: Amy Charkowski
Sent: Friday, September 23, 2011 11:28 AM
To: Ham, Jong
Subject: Re: Plant Disease

## (APPENDIX A continued)

```
Hi Jong Hyun,
Yes it is fine to include her chapter. I will have time to catch up on plant
disease things this weekend, so can provide an update then.
Best wishes -
Amy
Amy Charkowski
Dept. Plant Pathology
Univ. Wisconsin - Madison
```

Email correspondence between Dr. Jong Hyun Ham, advisor, and Dr. R. Michael Davis, Plant Disease senior editor-in-chief:

```
On Behalf Of jham@agcenter.lsu.edu
Sent: Wednesday, September 21, 2011 11:44 AM
To: R. Michael Davis
Subject: Plant Disease
9-21-2011
PDIS-09-11-0730-RE - A systematic study of the 16S-23S rRNA internal
transcribed spacer region, pglA, and ERIC-PCR and REP-PCR fingerprints
reveals that Xylella fastidiosa strains from pecan are part of X. fastidiosa
subsp. multiplex
Dear Dr. Davis:
Rebecca Melanson, the senior author of this manuscript and my graduate
student, will defend her master's thesis in November. We ask you a permit to
include a major portion of this manuscript into her thesis as a chapter.
Submission of this manuscript to Plant Disease will be clearly indicated on
the front page of the chapter and appropriate editing will be implemented to
the manuscript for fitting into the thesis.
We will be looking forward to hearing from you soon on this matter.
Sincerely,
Dr. Jong Hyun Ham
From: Mike Davis
Sent: Thursday, September 22, 2011 1:33 PM
To: Ham, Jong
Subject: RE: Plant Disease
Dr. Ham: Plant Disease allows and encourages this. Mike
```


## APPENDIX B: BACTERIAL STRAINS AND GENBANK ACCESSION NUMBERS USED IN THIS STUDY FOR PHYLOGENETIC ANALYSES

| Strain designation | $\begin{gathered} \hline \text { Plant } \\ \text { host } \\ \hline \end{gathered}$ | Geographical origin | $\begin{gathered} \text { GenBank } \\ \text { accession no } \\ \hline \end{gathered}$ | Accession no description | Reference |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Xylella fastidiosa |  |  |  |  |  |
| M12 | Almond | California | CP000941 | Complete genome ${ }^{\text {a }}$ | (Chen et al., 2010) |
| M23 | Almond | California | CP001011 | Complete genome | (Chen et al., 2010) |
| ALS1 | Almond | California | AF073240 | Partial ITS ${ }^{\text {b }}$ | (Hendson et al., 2001) |
| Tulare | Almond | California | AF073242 | Partial ITS | $\begin{aligned} & \text { (Hendson et al., } \\ & \text { 2001) } \end{aligned}$ |
| Contra Costa | Almond | California | AF073250 | Partial ITS | (Hendson et al., 2001) |
| Dixon | Almond | California | AF073251 | Partial ITS | (Hendson et al., 2001) |
| Fresno | Almond | California | DQ011259 | Partial ITS | (Hernandez- <br> Martinez et al., 2006) |
| AZ03 | Chitalpa | Arizona | EU714189 | Partial ITS | $\begin{aligned} & \text { (Randall et al., } \\ & 2009) \end{aligned}$ |
| CA01 | Chitalpa | California | EU714190 | Partial ITS | $\begin{aligned} & \text { (Randall et al., } \\ & 2009) \end{aligned}$ |
| NM02 | Chitalpa | New Mexico | EU714192 | Partial ITS | $\begin{aligned} & \text { (Randall et al., } \\ & 2009) \end{aligned}$ |
| 9 a c | Citrus | Brazil | AE003849 | Complete genome | $\begin{aligned} & \text { (Simpson et al., } \\ & 2000) \end{aligned}$ |
| CI. 52 | Citrus | Brazil | AF203393 | Partial ITS | (Mehta and Rosato, 2001) |
| CI. 11067 | Citrus | Brazil | AF237650 | Partial ITS | (Mehta and Rosato, 2001) |
| CI.X0 | Citrus | Brazil | AF237651 | Partial ITS | (Mehta and Rosato, 2001) |
| CO. 01 | Coffee | Brazil | AF203394 | Partial ITS | (Mehta and Rosato, 2001) |
| P3 | Coffee | Brazil | AY388464 | Partial ITS | $\begin{aligned} & \text { (Martinati et al., } \\ & \text { 2005) } \end{aligned}$ |
| ELM-1 | Elm | Washington | AY388468 | Partial ITS | (Martinati et al., 2005) |
| Temecula 1 | Grapevine | California | AE009442 | Complete genome | $\begin{aligned} & \text { (Van Sluys et al., } \\ & \text { 2003) } \end{aligned}$ |
| XF GB514 | Grapevine | Texas | CP002165 | Complete genome | $\begin{gathered} \text { (Schreiber IV et } \\ \text { al., 2010) } \end{gathered}$ |

(APPENDIX B continued)

| Strain designation | Plant host | Geographical origin | GenBank accession no | Accession no description | Reference |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\underline{\text { Xylella fastidiosa }}$ |  |  |  |  |  |
| PD 95-2 | Grapevine | Florida | AF073220 | Partial ITS | (Hendson et al., 2001) |
| PD 95-4 | Grapevine | Florida | AF073221 | Partial ITS | (Hendson et al., 2001) |
| P 95-9 | Grapevine | Florida | AF073222 | Partial ITS | (Hendson et al., 2001) |
| R116V3 | Grapevine | Florida | AF073223 | Partial ITS | $\begin{aligned} & \text { (Hendson et al., } \\ & \text { 2001) } \end{aligned}$ |
| R116V3-4 | Grapevine | Florida | AF073224 | Partial ITS | (Hendson et al., 2001) |
| Conn Creek | Grapevine | California | AF073225 | Partial ITS | (Hendson et al., 2001) |
| Santa Cruz | Grapevine | California | AF073229 | Partial ITS | (Hendson et al., 2001) |
| Preston Ranch | Grapevine | California | AF073232 | Partial ITS | (Hendson et al., 2001) |
| Moore Park | Grapevine | California | AF073236 | Partial ITS | (Hendson et al., 2001) |
| XF A-08 | Grapevine | Louisiana | JN092384 | $\begin{gathered} \text { Partial ITS } \\ p g l A^{\mathrm{c}} \end{gathered}$ | This study |
|  |  |  | JN092393 |  | This study |
| Acer macrophyla | Maple | California | AF073219 | Partial ITS | (Hendson et al., 2001) |
| MUL-1 | Mulberry | Massachusetts | AY388467 | Partial ITS | (Martinati et al., 2005) |
| 88-9 | Oak | Florida | AF073210 | Partial ITS | (Hendson et al., 2001) |
| 92-3 | Oak | Florida | AF073211 | Partial ITS | (Hendson et al., 2001) |
| 92-10 | Oak | Florida | AF073212 | Partial ITS | (Hendson et al., 2001) |
| OLS\#2 | Oak | Georgia | AF073213 | Partial ITS | (Hendson et al., 2001) |
| Stucky | Oak | Georgia | AF073214 | Partial ITS | (Hendson et al., 2001) |
| Ann1 | Oleander | California | AF073215 | Partial ITS | (Hendson et al., 2001) |
| PF1 | Oleander | California | AF073216 | Partial ITS | $\begin{aligned} & \text { (Hendson et al., } \\ & \text { 2001) } \end{aligned}$ |
| H44 | Oleander | California | AY603088 | Partial ITS | (Costa et al., 2004) |
| Riverside | Oleander | California | DQ011263 | Partial ITS | (HernandezMartinez et al., 2006) |

## (APPENDIX B continued)

| Strain designation | Plant host | Geographical origin | GenBank accession no | Accession no description | Reference |
| :---: | :---: | :---: | :---: | :---: | :---: |
| $\underline{\text { Xylella fastidiosa }}$ |  |  |  |  |  |
| Texas | Oleander | Texas | DQ011264 | Partial ITS | (HernandezMartinez et al., 2006) |
| XF Oleander | Oleander | Louisiana | JN092385 | $\begin{gathered} \text { Partial ITS } \\ p g l A \end{gathered}$ | This study |
|  |  |  | JN092394 |  | This study |
| 5S2 | Peach | Georgia | AF073206 | Partial ITS | (Hendson et al., 2001) |
| 5R1 | Peach | Georgia | AF073207 | Partial ITS | (Hendson et al., 2001) |
| 4S3 | Peach | Georgia | AF073208 | Partial ITS | (Hendson et al., 2001) |
| PE.PLS | Pear | Taiwan | AF203396 | Partial ITS | (Mehta and Rosato, 2001) |
| XF A-05 | Pecan | Louisiana | JN092378 | $\begin{gathered} \text { Partial ITS } \\ p g l A \end{gathered}$ | This study |
|  |  |  | JN092387 |  | This study |
| XF B-05 | Pecan | Louisiana | JN092379 | Partial ITS pglA | This study |
|  |  |  | JN092388 |  | This study |
| XF C-05 | Pecan | Louisiana | JN092380 | Partial ITS pglA | This study |
|  |  |  | JN092389 |  | This study |
| XF A-06 | Pecan | Louisiana | JN092381 | $\begin{gathered} \text { Partial ITS } \\ p g l A \end{gathered}$ | This study |
|  |  |  | JN092390 |  | This study |
| XF D-07 | Pecan | Louisiana | JN092383 | $\begin{gathered} \text { Partial ITS } \\ p g l A \end{gathered}$ | This study |
|  |  |  | JN092392 |  | This study |
| XF B-10 PWT-22 | Pecan Periwinkle | Louisiana Florida | JN092386 | Partial ITS | This study |
|  |  |  | AY388470 | Partial ITS | $\begin{aligned} & \text { (Martinati et al., } \\ & \text { 2005) } \end{aligned}$ |
| 2\#4 | Plum | Georgia | AF073209 | Partial ITS | (Hendson et al., 2001) |
| PL. 788 | Plum | Georgia | AF203395 | Partial ITS | (Mehta and Rosato, 2001) |
| RGW-R | Ragweed | Florida | AY388469 | Partial | $\begin{gathered} \text { (Martinati et al., } \\ \text { 2005) } \end{gathered}$ |
| XF A-07 | Sycamore | Louisiana | JN092382 | Partial ITS pglA | This study |
|  |  |  | JN092391 |  | This study |
| $\underline{\text { Xanthomonas campestris pv. campestris }}$ |  |  |  |  |  |
| LMG 568 | -- | -- | AF209755 | Complete ITS | (Gonçalves and <br> Rosato, 2002) |

## (APPENDIX B continued)

${ }^{\text {a }}$ Accession numbers for the complete genome of strains is listed. M12, an Almond Leaf Scorch (ALS) strain, is a X. fastidiosa subsp. multiplex strain; M23, an ALS strain, and Temecula1 and XF GB514, Pierce's Disease strains, are $X$. fastidiosa subsp. fastidiosa strains; and 9a5c, a citrus variegated chlorosis strain, is a $X$. fastidiosa subsp. pauca strain. Only a small portion of these genomes was extracted from each genome for phylogenic analysis.
${ }^{6}$ ITS $=$ intergenic transcribed spacer region between the 16 S and 23 S ribosomal subunits described in NCBI's GenBank as the 16S-23S ribosomal RNA intergenic spacer.
${ }^{\text {c }} p g l A$ was designated as the sequence description for the strains used in this study based on the homology with the polygalacturonase gene in the complete genome sequences of $X$. fastidiosa strains 9a5c, Temecula1, M12, M23, and XF GB514 available in NCBI's GenBank.

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## (APPENDIX B continued)

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## APPENDIX C: RAXML PROGRAMS FOR MAXIMUM LIKELIHOOD SEARCHES OF 16S-23S ITS AND DATASETS

RAxML program to run ITS sequence analysis
raxml -f a -x 12345 -p 12345 -\# 1000 -m GTRMIX -s XF_ITS_curedml -n XFITS_cured_outfile
pause
RAxML program to run pglA sequence analysis
raxml -f a -x 12345 -p 12345 -\# 1000 -m GTRMIX -s XF_PGLA -n XF_PGLA_outfile
pause

## APPENDIX D: MRBAYES 3.1 PROGRAMS FOR MARKOV CHAIN MONTE CARLO (MCMC) SEARCHES OF 16S-23S ITS, PGLA, ERIC-PCR, REP-PCR, AND COMBINED DATASETS

*Note: Sequence data was omitted from this appendix for 16S-23S ITS, pglA, and 16S-23S, $p g l A$, REP-PCR, and ERIC-PCR combined dataset programs, but was included in the actual MCMC searches.

## MrBayes 3.1 program to run 16S-23S ITS MCMC Search* <br> BEGIN mrbayes;

```
set autoclose=yes nowarn=yes;
charset ITS = 1-503;
partition ITS = 1: ITS;
set partition = ITS;
lset applyto=(1) nst=6 rates=gamma;
lset coding = all;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
```

usertree =
(Pecan XF A05, (( (Pecan_XF B05, Pecan_XF A06), (Sycamore_XF_A07,Pecan_XF B10)), (
Pecan_XF_D07, (( (AY388470_Periwinkle_PWT_22,AY388469_Ragweed_RGW_R), (( (EU71418
9_Chitalpa_AZ03,EU714192_Chitalpa_NM02), EU714190_Chitalpa_CA01), (()((DQ011263
O Oleander_Texas, DQ011264_Oleander_Riverside), (Olēander_XF_Oleander,AY603088_0

pevine_R116V3,AF073232_Grapevine_Preston_Ranch ), ((AF073̄220_Grapevine_PD_95_2,
(AF073221_Grapevine_PD_95_4,AF073219_Maple_Acer_macrophyla)), (AF073240_Almond
_ALS1, (( ( $A F 073236$ Grapevine_Moore_Park, AF $07322 \overline{9}$ _Grapevine_Santa_Cruz), AF0732


8468 Elm_ELM_1) ) ), ((AF073251_Almond_Dixon, (( (AF073250_Almond_Contra_Costa, AF0
73206 Peach_5S2),AF073207_Peach_5R1),AF073208_Peach_4S3)), (( (AF073212_Oak_92
10, AF $\overline{0} 73213$ _Oak_OLS2) , AF073214_Oak_Stucky), (AF073210_Oak_88_9, AF073211_Oak_9



mcmcp startingtree=user ngen=100000000 temp=0.25 nruns=4 samplefreq=10
printfreq=500 nchains=4 savebrlens=yes
filename=XF_ITS_cured.nex stoprule=yes stopval=0.02;
[sump filename $=$ XF ITS cured.nex;
sumt filename $=$ XF_ITS_cured.nex; ]
END;

## MrBayes 3.1 program to run $p g l A$ MCMC Search* BEGIN mrbayes;

```
set autoclose=yes nowarn=yes;
charset PGLA = 1-1636;
```


## (APPENDIX D continued)

```
partition PGLA = 1: PGLA;
set partition = PGLA;
lset applyto=(1) nst=6 rates=gamma;
lset coding = all;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
mcmcp ngen=100000000 temp=0.25 nruns=4 samplefreq=100 printfreq=5000
nchains=4 savebrlens=yes
filename=XF_PGLA.nex stoprule=yes stopval=0.02;
[sump filename = XF_PGLA.nex;
sumt filename = XF_PGGLA.nex;]
END;
```


## MrBayes 3.1 program to run ERIC-PCR MCMC Search

BEGIN DATA;
DIMENSIONS NTAX=10 NCHAR=10;
FORMAT DATATYPE=standard MISSING=? GAP=- interleave=yes;
MATRIX
Pecan_XF_A05 0111011010
Pecan_XF_B05 0111011010
Pecan_XF_C05 0111011010
Pecan_XF_A06 0111011010
Sycamōre_XF_A07 0111011010
Pecan_XF_D0̄ 0111011010
Grapevine_XF_A08 1011111100
Oleander_X ${ }^{\text {F_O}} 1$ Oander 1000111101
Pecan XF ${ }^{-}{ }^{\text {A1 }} \overline{0} \quad 0111011010$
Pecan_XF_B10 0111011010
;
END;
BEGIN mrbayes;
set autoclose=yes nowarn=yes;
charset REP = 1-10;
partition REP = 1: REP;
set partition = REP;
lset coding = noabsencesites;
lset coding = all;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
mcmcp ngen=100000000 temp=0.25 nruns=4 samplefreq=10 printfreq=500 nchains=4
savebrlens=yes
filename=XF_ERIC.nex stoprule=yes stopval=0.01;
[sump filename $=X F$ ERIC.nex;

## (APPENDIX D continued)

```
sumt filename = XF_ERIC.nex;]
END;
```

MrBayes 3.1 program to run REP-PCR MCMC Search
BEGIN DATA;
DIMENSIONS NTAX=10 NCHAR=9;
FORMAT DATATYPE=standard MISSING=? GAP=- interleave=yes;
MATRIX
Pecan_XF_A05 110001010
Pecan_XF_B05 110001010
Pecan_XF-C05 110001010
Pecan_XF_A06 100011010
Sycamore_XF_A07 100011010
Pecan_XF_D07 110001010
Grapevine_XF_A08 001001000
Oleander $\bar{X} F$ Oleander 100101100
Pecan_XF_A1 $\overline{0} 110001001$
Pecan_XF_B10 110001010
;
END;
BEGIN mrbayes;
set autoclose=yes nowarn=yes;
charset REP = 1-9;
partition REP = 1: REP;
set partition = REP;
lset coding = noabsencesites;
lset coding = all;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
mcmcp ngen=100000000 temp=0.25 nruns=4 samplefreq=10 printfreq=500 nchains=4
savebrlens=yes
filename=XF_REP.nex stoprule=yes stopval=0.01;
[sump filename = XF_REP.nex;
sumt filename $=$ XF_REP.nex; $]$
END;

MrBayes 3.1 program to run REP-PCR and ERIC-PCR Combined Dataset MCMC Search
BEGIN DATA;
DIMENSIONS NTAX=10 NCHAR=19;
FORMAT DATATYPE=standard MISSING=? GAP=- interleave=yes;
MATRIX
Pecan_XF_A05
1100010100111011010
Pecan_XF_B05
1100010100111011010

## (APPENDIX D continued)

```
Pecan_XF_C05 1100010100111011010
Pecan_XF-A06 1000110100111011010
Sycamōre_XF_A07 1000110100111011010
Pecan_XF_}\mp@subsup{}{-}{\prime}0\overline{7
Grapevine_XF_A08 0010010001011111100
Oleander_XF_Oleander 1001011001000111101
Pecan_XF'A1\overline{0}}110001001011101101
Pecan_XF_B10 1100010100111011010
;
END;
BEGIN mrbayes;
set autoclose=yes nowarn=yes;
charset REP = 1-19;
partition REP = 1: REP;
set partition = REP;
lset coding = noabsencesites;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
mcmcp ngen=100000000 temp=0.25 nruns=4 samplefreq=10 printfreq=500 nchains=4
savebrlens=yes
filename=XF REP-ERIC.nex stoprule=yes stopval=0.01;
[sump filename = XF_REP-ERIC.nex;
sumt filename = XF_REP-ERIC.nex;]
END;
```


## MrBayes 3.1 program to run 16S-23S ITS, pglA, and REP-PCR and ERIC-PCR Combined

 Dataset MCMC Search*BEGIN mrbayes;

```
set autoclose=yes nowarn=yes;
charset ITS = 1-503;
charset PGLA = 504-2139;
charset REP-PCR = 2140-2148;
charset ERIC-PCR = 2149-2158;
partition IPRE = 4: ITS, PGLA, REP-PCR, ERIC-PCR;
set partition = IPRE;
lset applyto=(1,2) nst=6 rates=gamma;
lset coding = all;
unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all);
prset applyto=(all) ratepr=variable;
usertree =
    (Pecan_XF_A05,(((Pecan_XF_B05,Pecan_XF_A06),(Sycamore_XF_A07,Pecan_XF_B10)),(
Pecan_\overline{XF_D}07,(((AY3884\overline{70_Periwinkle_PWT_22,AY388469_Rägweed_RGW_R) ,(()}(EU71418
9_Chītalp
    _Oleander_Texas,DQ011264_Oleander_Riverside),(Ol\overline{eander_XF_Oleander,AY603088_0}0
leander_H44)),(AF073216_Oleander_PF1,AF073215_Oleander_Ann1)),(((AF073223_Gra
```


## (APPENDIX D continued)

```
pevine R116V3,AF073232_Grapevine_Preston_Ranch),((AF073220_Grapevine_PD_95_2,
(AF073\overline{2}21 Grapevine PD-95 4,AF07\overline{3}219 Maple Acer macrophyla)), (AF0732\overline{4}0}\overline{\textrm{A}}\textrm{l
ALS1,(((\overline{(AF073236 Ḡra\overline{p}evíne Moore Pārk,AF\overline{0}7322\overline{9}}\mathrm{ Grapevine Santa Cruz),AF0732}
25 Grapevine Conn Creek),AF073224_Grapevine_R118V3_4),AF073242_Almond_Tulare)
))),((AY388467_Mulberry_MUL_1,(DQ011259_Almond_Fresno,Grapevine_XF_A08)),AY38
8468 Elm ELM 1))),((AF073251 Almond Dixon,(((AF073250 Almond Contra Costa,AF0
7320\overline{6}}\mathrm{ Pē̄ch 5
10,AF\overline{0}73213 Oak OLS2),AF073214 Oak Stucky), (A\overline{F}073210
_3))))),((AF203395_Plum_PL_788,((AF203393_Citrus_CI_52,AF203393_Coffee_CO_01
),(AF237651_Citrus_CI_X0,AF237650_Citrus_CI_11067))),(AF209755_Xanthomonas_ca
mpestris_LMG568,AF203396_Pear_PE_PLS))))),Pecan_XF_C05);
mcmcp startingtree=user ngen=5000000 temp=0.15 nruns=4 samplefreq=100
printfreq=5000 nchains=4 savebrlens=yes
filename=XF_IPRE.nex stoprule=yes stopval=0.02;
[sump filename = XF IPRE.nex;
sumt filename = XF I
END;
```


# APPENDIX E: 16S-23S ITS NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA 

>XF_A-05_ITS [organism=Xylella fastidiosa][strain=XF A-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer]
AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGTC TTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTAT GTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGGG GTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGGT TAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTAT ATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCGT TCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTCTCATTGGAAGC CTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTTATATGGTCAA GCGAATAAGCGCACACGG
>XF_B-05_ITS [organism=Xylella fastidiosa][strain=XF B-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer] GAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGA ATATAATTGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGG TTTGGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTT GCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTA GCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCC ACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAG CTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTC TCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGT TATATGGTCAAGCGAATAAGCGCACACGGTGGA
>XF_C-05_ITS [organism=Xylella fastidiosa][strain=XF C-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer] CAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGT CTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTA TGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGG GGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGG TTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTA TATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCG TTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTCTCATTGGAAG CCTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTTATATGGTCA AgCGAATAAGCGCACACGG
>XF_A-06_ITS [organism=Xylella fastidiosa][strain=XF A-06][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer]
GAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGA ATATAATTGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGG TTTGGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTT

## (APPENDIX E continued)

GCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTA GCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCC ACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAG CTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTC TCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGT TATATGGTCAAGCGAATAAGCGCACACG
>XF_A-07_ITS [organism=Xylella fastidiosa][strain=XF A-07][host=sycamore][country=USA: Louisiana][note $=16 \mathrm{~S}-23 \mathrm{~S}$ intergenic transcribed spacer]
AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGTC TTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTAT GTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGGG GTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGGT TAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTAT ATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCGT TCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTCTCATTGGAAGC CTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTTATATGGTCAA GCGAATAAGCGCACACGGTG
>XF_D-07_ITS [organism=Xylella fastidiosa][strain=XF D-07][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer]
AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGTC TTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTAT GTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGGG GTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGGT TAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTAT ATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCGT TCTTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTCTCATTGGAAGC CTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTTATATGGTCAA GCGAATAAGCGCACACGGTGGAT
>XF_A-08_ITS [organism=Xylella fastidiosa][strain=XF A-08][host=grapevine][country=USA: Louisiana][note $=16 \mathrm{~S}-23 \mathrm{~S}$ intergenic transcribed spacer]
AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGTC TTACCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTAT GTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGGG GTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGGT TAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTAT ATCAATTATTCTGAATGTAGTTTGCGCATTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCGTT CTTTTATAATTTGATGATGTAGCAAGCGTTTGAAATTTTTATTAAAAATTTCTCATTTGAAGCC TTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTGGGGTTATATGGTCAAG CGAATAAGCGCACAC

## (APPENDIX E continued)

>XF_Oleander_ITS [organism=Xylella fastidiosa][strain=XF Oleander][host=oleander] [country=USA: Louisiana][note=16S-23S intergenic transcribed spacer]
GAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGA ATATAATTGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGG TTTGGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTT GCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATCAAAGTATTTATGGGTCTGTA GCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCC ACCAATGTTATATCAATTATTCTGAATGTATTTTGCGCATTTTTATGCTTATCAGCCTTGGAGC TGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAATTTTTATTAAAAATTTCT CATTTGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTT ATATGGTCAAGCGAATAAGCGCACACGGT
>XF_B-10_ITS [organism=Xylella fastidiosa][strain=XF B-10][host=Carya illinoinensis (pecan) cultivar Desirable][country=USA: Louisiana][note=16S-23S intergenic transcribed spacer] GGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAATTGTCTT ATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTTGGGTTTATGT TGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTTTGCAAGCAGGGGGT CGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGTCTGTAGCTCAGGTGGTTA GAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCTCCCAGACCCACCAATGTTATAT CAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTTATCAGCCTTGGAGCTGTGAAGCGTTC TTTTATAATTTGATGATGTAGCAAGCGTTTGAACTTTTTATTAATAATTTCTCATTGGAAGCCT TAAGTGACAATGTTTATCCATTGTCTTGTAGATTTTGAGGCGACTTTGGGTTATATGGTCAAGC GAATAAGCGCACACG

# APPENDIX F: CLUSTALW2 ALIGNMENT AND SUMMARY OF THE 16S-23S ITS NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA 

## ClustalW2 Alignment

XF A-05
$\mathrm{XF}^{-} \mathrm{B}-05$
XF_C-05
XF_A-06
XF A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
XF_B-10
XF_A-08
XF_Oleander

XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
XF_A-07
XF-D-07
XF B-10
XF_A-08
XF_Oleander

XF A-0 5
XF_B-05
$\mathrm{XF}_{-}^{-} \mathrm{C}-05$
XFA-06
XF_A-07
XF_D-07
$\mathrm{XF}-\mathrm{B}-10$
$\mathrm{XF}_{-}^{-} \mathrm{A}-08$
XF_Oleander

XF A-0 5
$\mathrm{XF}^{-} \mathrm{B}-05$
XF_C-05
XF_A-06
XF A-07
XF_D-07
XF_B-10
XF_A-08
XF_Oleander

XF_A-05
$X F^{-} B-05$
XF_C-05
XF_A-06
XF_A-07
XF-D-07
XF_B-10
XF_A-08
XF_Oleander

AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60 AAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTTTGAGTATGGTGAATATAAT 60

TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120
TGTCTTACCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120 TGTCTTATCAGGCGTCCTCACAAGTTACTTGCATTCAGGGTTTGATGTTGGCATAGGTTT 120

GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180 GGGTTTATGTTGGCGATTTTTGTTCTGGGGGCCTTAGCTCAGCTGGTAGAGCACCTGCTT 180


TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATGAAAGTATTTATGGGT 240 TGCAAGCAGGGGGTCGTCGGTTCGATCCCGACAGGCTCCACCATCAAAGTATTTATGGGT 240


CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300 CTGTAGCTCAGGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGGTTCGAGTCCT 300


## (APPENDIX F continued)

XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
XF_A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
$\mathrm{XF}^{-} \mathrm{B}-10$
XF_A-08
XF_Oleander

XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
$\mathrm{XF}^{-} \mathrm{A}-06$
XF_A-07
XF_D-07
XF-B-10
$\mathrm{XF}^{-} \mathrm{A}-08$
XF_Oleander

XF A-05
$\mathrm{XF}^{-}{ }^{-}$- 05
XF_C-05
XF_A-06
XF A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
XF_B-10
XF_A-08
XF_Oleander

XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
XF_A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
XF_B-10
XF_A-08
XF_Oleander

CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTGGTTTGCGCATTTTTTATGCTT 360 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTAGTTTGCGCATTTTT-ATGCTT 359 CCCAGACCCACCAATGTTATATCAATTATTCTGAATGTATTTTGCGCATTTTT-ATGCTT 359

ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAC 420 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAA 419 ATCAGCCTTGGAGCTGTGAAGCGTTCTTTTATAATTTGATGATGTAGCAAGCGTTTGAAA 419 ****************************************************************

TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAATAATTTCTCATTGGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 480 TTTTTATTAAAAATTTCTCATTTGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 479 TTTTTATTAAAAATTTCTCATTTGAAGCCTTAAGTGACAATGTTTATCCATTGTCTTGTA 479
$\star \star \star \star \star \star \star \star * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *$
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 528
GATTTTGAGGCGACTTGGGGTTATATGGTCAAGCGAATAAGCGCACAC 527
GATTTTGAGGCGACTTTGGGTTATATGGTCAAGCGAATAAGCGCACAC 527


ClustalW2 Summary

| SeqA | Name | Length | SeqB | Name | Length | Score |
| ---: | :--- | ---: | ---: | :--- | ---: | ---: |
| 1 | XF_A-05 | 528 | 2 | XF_B-05 | 528 | 100 |
| 1 | XF_A-05 | 528 | 3 | XF_C-05 | 528 | 100 |
| 1 | XF_A-05 | 528 | 4 | XF_A-06 | 528 | 100 |
| 1 | XF_A-05 | 528 | 5 | XF_A-07 | 528 | 100 |
| 1 | XF_A-05 | 528 | 6 | XF_D-07 | 528 | 100 |
| 1 | XF_A-05 | 528 | 7 | XF_A-08 | 527 | 98 |

(APPENDIX F continued)

| $\operatorname{Seg} A$ | Name | Length | SegB | Name | Length | Score |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | XF_A-05 | 528 | 8 | XF_Oleander | 527 | 98 |
| 1 | XF_A-05 | 528 | 9 | XF_B-10 | 528 | 100 |
| 2 | XF_B-05 | 528 | 3 | XF_C-05 | 528 | 100 |
| 2 | XF_B-05 | 528 | 4 | XF_A-06 | 528 | 100 |
| 2 | XF_B-05 | 528 | 5 | XF_A-07 | 528 | 100 |
| 2 | XF_B-05 | 528 | 6 | XF_D-07 | 528 | 100 |
| 2 | XF_B-05 | 528 | 7 | XF_A-08 | 527 | 98 |
| 2 | XF_B-05 | 528 | 8 | XF_Oleander | 527 | 98 |
| 2 | XF_B-05 | 528 | 9 | XF_B-10 | 528 | 100 |
| 3 | XF_C-05 | 528 | 4 | XF_A-06 | 528 | 100 |
| 3 | XF_C-05 | 528 | 5 | XF_A-07 | 528 | 100 |
| 3 | XF_C-05 | 528 | 6 | XF_D-07 | 528 | 100 |
| 3 | XF_C-05 | 528 | 7 | XF_A-08 | 527 | 98 |
| 3 | XF_C-05 | 528 | 8 | XF_Oleander | 527 | 98 |
| 3 | XF_C-05 | 528 | 9 | XF_B-10 | 528 | 100 |
| 4 | XF_A-06 | 528 | 5 | XF_A-07 | 528 | 100 |
| 4 | XF_A-06 | 528 | 6 | XF_D-07 | 528 | 100 |
| 4 | XF_A-06 | 528 | 7 | XF_A-08 | 527 | 98 |
| 4 | XF_A-06 | 528 | 8 | XF_Oleander | 527 | 98 |
| 4 | XF_A-06 | 528 | 9 | XF_B-10 | 528 | 100 |
| 5 | XF_A-07 | 528 | 6 | XF_D-07 | 528 | 100 |
| 5 | XF_A-07 | 528 | 7 | XF_A-08 | 527 | 98 |
| 5 | XF_A-07 | 528 | 8 | XF_Oleander | 527 | 98 |
| 5 | XF_A-07 | 528 | 9 | XF_B-10 | 528 | 100 |
| 6 | XF_D-07 | 528 | 7 | XF_A-08 | 527 | 98 |
| 6 | XF_D-07 | 528 | 8 | XF_Oleander | 527 | 98 |
| 6 | XF_D-07 | 528 | 9 | XF_B-10 | 528 | 100 |
| 7 | XF_A-08 | 527 | 8 | XF_Oleander | 527 | 99 |
| 7 | XF_A-08 | 527 | 9 | XF_B-10 | 528 | 98 |
| 8 | XF_Oleander | 527 | 9 | XF_B-10 | 528 | 98 |

## APPENDIX G: BLAST SEARCH OF 16S-23S ITS NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA

BLAST Query Results: XF A-05 (Identical to all LA pecan and sycamore ITS sequences)

| Accession Description |  |  |  | Query |  | Max |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | score | score | coverage | value | ident |
| CP000941.1 | Xylella fastidiosa M12, complete genome | 974 | 1948 | 99\% | 0.0 | 100\% |
| AE003849.1 | Xylella fastidiosa 9asc, complete genome | 952 | 1904 | 99\% | 0.0 | 99\% |
| CP002165:1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | 233 | 1867 | 99\% | 0.0 | 98\% |
| CP001011.1 | Xylella fastidiosa M23, complete genome | 933 | 1867 | 99\% | 0.0 | 98\% |
| AE0094421 | Xylella fastidiosa Temecula 1 , complete genome | 933 | 1867 | 99\% | 0.0 | 98\% |
| AF073215.1 | Xylella fastidiosa strain Arn1 16S-23S ribosomal RNA intergenic spacer, partial sequence | 883 | 883 | 96\% | 0.0 | 98\% |

BLAST Query Results: XF A-08 (LA grapevine strain)

| Accession Description |  | Max <br> score | Total score | Query coverage | E <br> value | $\frac{\text { Max }}{\text { ident }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CP002165.1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | 972 | 1944 | 99\% | 00 | 100\% |
| CP001011.1 | Xylelia fastidiosa M23, complete genome | 972 | 1944 | 99\% | 0.0 | 100\% |
| AED09442.1 | Xylella fastidiosa Temecula 1, complete genome | 972 | 1944 | 99\% | 0.0 | 100\% |
| CP000941.1 | Xylella fastidiosa M12, complete genome | 933 | 1867 | 99\% | 00 | 98\% |
| AE003849.1 | Xylella fastidiasa 9a5c, complete genome | 911 | 1823 | 99\% | 0.0 | 97\% |
| AF073215.1 | Xylella fastidiosa strain Ann1 16S-23S nibosomal RNA intergenic spacer, partial sequence | 898 | 898 | 96\% | 0.0 | 98\% |

BLAST Query Results: XF Oleander (LA oleander strain )

| Accession Description |  | $\frac{\text { Max }}{\text { score }}$ | Total score | Query coverage | E value | $\frac{\text { Max }}{\text { ident }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CP002165.1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | 950 | 1900 | 99\% | 0.0 | 99\% |
| CP001011.1 | Xylella fastidiosa M23, complete genome | 950 | 1900 | 99\% | 0.0 | 99\% |
| AE0094421 | Xylella fastidiosa Temecula1, complete genome | 950 | 1900 | 99\% | 0.0 | 99\% |
| CP000941.1 | Xylella fastidiosa M12, complete genome | 933 | 1867 | 99\% | 0.0 | 98\% |
| AF073215.1 | Xylella fastidiosa strain Arn1 16S-23S nibosomal RNA intergenic spacer, partial sequence | 920 | 920 | 96\% | 0.0 | 99\% |
| AE003849.1 | Xylella fastidiosa 9a5c, complete genome | 911 | 1823 | 99\% | 0.0 | 97\% |

# APPENDIX H: PGLA NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA 

$>$ XF_A-05_PGLA [organism=Xylella fastidiosa][strain=XF A-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGCATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TtGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_B-05_PGLA [organism=Xylella fastidiosa][strain=XF B-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACgACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACT GgCGGTAAGCATGCTCGGCAGCGTACCtGgtGGGACcTGGCCTATCAGaACAAGCGCCAtgCac tGCATCAaCAGGTACCaCGGTTGATCCAAATCAGGGGCGGTAAcGacTTCACGTTGTaCCGTGT tGCCATCGAaAATGCACcTAAcTTCCATGTGGTTGcTGATACGGTCAGCGGTGTCACCGTATGG

## (APPENDIX H continued)

GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATcA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_C-05_PGLA [organism=Xylella fastidiosa][strain=XF C-05][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACgACAAAgGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCaA CACCACCGGTAGCGGTATCGTgGGTGGGGGTGTGAtTGATGGGCGTGGCgGtAGCATTTTGACT GGCGGTAAGCATGCTCGGCAGCGTAcctGGTGGGACcTGgCCTATCAGAACAAGCGCCATGCAc TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACaCGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA

## (APPENDIX H continued)

>XF_A-06_PGLA [organism=Xylella fastidiosa][strain=XF A-06][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGCATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_A-07_PGLA [organism=Xylella fastidiosa][strain=XF A-07][host=sycamore][country= USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGAtTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGCATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAgAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG

## (APPENDIX H continued)

CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_D-07_PGLA [organism=Xylella fastidiosa][strain=XF D-07][host=Carya illinoinensis (pecan) cultivar Cape Fear][country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCGGTGAA AACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCACAGGCTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTGCACTTGAAATCTAGGGTGACGTTG TGGATTGACGATGGCGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGCATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGTTGAGTCTGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTTGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_A-08_PGLA [organism=Xylella fastidiosa][strain=XF A-08][host=grapevine][country= USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGCGGCA

## (APPENDIX H continued)

CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTATCGGTGAA AACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGCTCCATTGACGCACTCGATGCCAATCCAAAGGTTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTGCACTTGAAATCTGGGGTGACGTTG TGGATTGACGAGGGTGTTACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTCATTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGTATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTTAGCGGTGTCACCGCATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGATATGGAAGT CAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGTTTGCAGATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGCTGAGCATGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTGTCGTATTCGCCAATCCGCAGGCAGT GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCA ATTTTTCCAGCGTATTTCCTGATTCGCCTATCTAA
>XF_Oleander_PGLA [organism=Xylella fastidiosa][strain=XF Oleander][host=oleander] [country=USA: Louisiana][note=pgla]
ATGAACCTTGACCGTTTCTTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGCGGCA CTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTATCTGTGAA AACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACGGTATTGCCTGCG CGCCTGACTCCTAAACATGGCTCCATTGACGCACTTGATGCCAATCCAAAGGTTTCCAAGCCTG ATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGTAGCGCGGTCAAACTAGTGAT CGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTGCACTTGAAATCTGGGGTGACGTTG TGGATTGACGATGGTGTCACGCTGTTTGCATCGCGCAACCCCAAGGATTACGACAAAGGCAATG GTACGTGCGGTACTGCAACGTCAACTCATGAGTTCAGTTGTATGCCTTTGATCTCCGCGATCAA CACCACCGGTAGCGGTATCGTGGGTGGGGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACT GGCGGTAAGTATGCTCGGCAGCGTACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCAC TGCATCAACAGGTACCACGGTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGT TGCCATCGAAAATGCACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGCATGG GGCATCCGCATCCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTA CCACGCCGGATGTTGTGACGCCGGCGACATGCTTTACCCCCGACACGGTCAAGAACACCGATGG CTTTGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGAC CACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAACCACT TTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGATATGGAAGT

## (APPENDIX H continued)

CAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGCTTGCATATGAAATCTGAT GCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATGCATGCGTCGTCTGAAAC GTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAATTCTTATCCACTCTTCAAAAA TATTGTGTTGCAAGATATTCATGTGCTTGAAAGCCCTGTCTTTGGTGCTGGTCAGCTGCTTTTC ATGGGGATCCTGGGCAGTGGGAATAATCTGCCAATGACGCTGAGCATGGATAACGTCGTATTCG ACGGTTTTTTGCCAACATTGATAGCGCCTCCCAGCAGTATCGTATTCGCCAATCCGCAGGCAGC GCATTTTCACTTCGGTCCCGGTCCGGTCAGTTTCGCTCCTTTGATTACCCCCTCTGTTGCTTAC GATGTGACTGTGAGTGGAAGTCCAGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCA ATTTTTCCAGCATATTTCCTGATTCGCCTATCTAA

# APPENDIX I: CLUSTALW2 ALIGNMENT AND SUMMARY OF THE PGLA NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA AND USED IN THIS STUDY 

## ClustalW2 Alignment

XF A-08
M2 $\overline{3}$ ALSD
Temecula1_PD
XF GB514
XF ${ }^{-}$Oleander
XF_A-05
XF_B-05
XF-C-05
XF_A-06
XF A-07
XF-D-07
M12_ALSD
9a5c_CVC

XF A-08
M23_ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
XF-B-05
XF C-05
$X F^{-} A-06$
XF_A-07
XF D-07
M1 $\overline{2}$ ALSD
9a5c_CVC

| XF_A-08 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 |
| :---: | :---: |
| M23_ALSD | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 |
| Temecula1_PD | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 |
| XF_GB514 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 |
| XF_Oleander | ATGAACCTTGACCGTTTCTTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_A-05 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_B-05 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_C-05 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_A-06 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_A-07 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| XF_D-07 | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| M12_ALSD | ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 |
| 9a5c_CVC | ATGAACCTTGACCGTTTCCTCCCCCTAGTCTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 <br>  |
| XF_A-08 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 |
| M23_ALSD | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 |
| Temécula1_PD | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 |
| XF_GB514 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 |
| XF_Oleander | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_A-05 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_B-05 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_C-05 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_A-06 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_A-07 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| XF_D-07 | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| M12 _ALSD | GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 |
| 9a5 $\overline{\text { c }}$-CVC | GGCACTGCTGATGTCTTTCGAGGATCACAGCCTTCCCTATCCGATCTTTCCGAGCCGGTA 120 |
|  |  |

ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 GAACCIGACCGIITCCICCCCCIAGTGIIIGGACIGCACIGIIITGCCGCAAIGGGC-60 ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 A ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 ATGAACCTTGACCGTTTCCTCCCCCTAGTGTTTGGACTGCATTGTTTTGCCGCAATGGGC 60 ATGAACCTTGACCGTTTCCTCCCCCTAGTCTTTGGACTGCACTGTTTTGCCGCAATGGGC 60 GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCAGATCTTTCCGAGCCGGTA 120 GgivigAigicilicgAgIgICACAGCIGICcICAICAGAICITHCCGAGCCGGIA 120 GGCACTCO 120 GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120
 GCA GGCACTGCTGATGTCTTTCGAGTGTCACAGCTGTCCTCATCCGATCTTTCCGAGCCGGTA 120 GGCACTGCTGATGTCTTTCGAGGATCACAGCCTTCCCTATCCGATCTTTCCGAGCCGGTA 120

TCGGTGAAAACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG 180 TCGGTGAAAACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCTGTGAAAACGCTATGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG TCGGTGAAAACGCTCTGGGGCGAGGTGCAACGTCCCAGCCTACCTACCCACGTGTGTACG $\star * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *$

GTATTGCCTGCGCGCCTGACTCCTAAACATGGCTCCATTGACGCACTCGATGCCAATCCA GTATTGCCTGCGCGCCTGACTCCTAAACATGGCTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGCTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGCTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGCTCCATTGACGCACTTGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTAAACATGGTTCCATTGACGCACTCGATGCCAATCCA 240 GTATTGCCTGCGCGCCTGACTCCTGAACATGGCTCCCTTGACGCACTCGATGCCAATCCA 240

## (APPENDIX I continued)

XF_A-08
M23_ALSD
Temecula1_PD
XF_GB514
XF_Oleander
XF_A-05
XF_B-05
XF_C-05
XF_A-06
XF_A-07
XF_D-07
M12_ALSD
9a5C_CVC

XF A-08
M23_ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
$X F^{-} B-05$
XF_C-05
$X F^{-} A-06$
XF_A-07
XF D-07
M1 $\overline{2}$ _ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ ALSD
Temecula1_PD
XF_GB514
XF-Oleander
XF_A-05
XF-B-05
XF-C-05
XF_A-06
XF A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
XF-B-05
XF_C-05
$X F^{-} A-06$
XF_A-07
XF $\mathrm{D}-07$
M12_ALSD
9a5c_CVC

AAGGTTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 AAGGTTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 AAGGTTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 AAGGTTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 AAGGTTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CAGGCTTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATTGATGATTGTCCCGCCGGT 300 CGGGCCTCCAAGCCTGATACCAAGCGTTTACAGGATGCCATCGATGATTGTCCCGCCGGT 300

AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATCGACAGTCATCGCAAATCCGGCTTTTTGAGCGGGCCGTTG 360 AGCGCGGTCAAACTAGTGATGGATAGTCATCGCAAATCCGGCTTTTTGAGTGGGCCGTTG 360
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CACTTGAAATCTGGGGTGACGTTGTGGATTGACGAGGGTGTTACGCTGTTTGCATCGCGC 420 CACTTGAAATCTGGGGTGACGTTGTGGATTGACGAGGGTGTTACGCTGTTTGCATCGCGC 420 CACTTGAAATCTGGGGTGACGTTGTGGATTGACGAGGGTGTTACGCTGTTTGCATCGCGC 420 CACTTGAAATCTGGGGTGACGTTGTGGATTGACGAGGGTGTTACGCTGTTTGCATCGCGC 420 CACTTGAAATCTGGGGTGACGTTGTGGATTGACGATGGTGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTAGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGC 420 CACTTGAAATCTGGGGTGACGTTGTGGATTGACGATGGCGTCACGCTGTTTGCATCGCGT 420
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AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGATTACGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACTCATGAG 480 AACCCCAAGGACTATGACAAAGGCAATGGTACGTGCGGTACTGCAACGTCAACCTATGAG 480

[^1]
## (APPENDIX I continued)

XF_A-08
M23_ALSD
Temecula1_PD
XF_GB514
XF_Oleander
XF_A-05
XF_B-05
XF-C-05
XF_A-06
XF_A-07
XF_D-07
M12_ALSD
9a5C_CVC

XF A-08
M23_ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
$X F^{-} B-05$
XF_C-05
$X F^{-} A-06$
XF_A-07
XF D-07
M1 $\overline{2}$ ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ ALSD
Temecula1_PD
XF_GB514
XF-Oleander
XF_A-05
XF_B-05
XF-C-05
XF_A-06
XF A-07
XF ${ }^{-}$D-07
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF $\mathrm{A}-05$
XF-B-05
XF_C-05
$X F^{-} A-06$
XF_A-07
XF $\mathrm{D}-07$
M12_ALSD
9a5c_CVC

TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCTTT-GATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 539 TTCAGTTGTATGCCATTAGATCTCCGCGATCAACACCACCGGTAGCGGTATCGTGGGTGG 540

GGGTGTCATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGTATGCTCGGCAGCG GGGTGTCATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGTATGCTCGGCAGCG GGGTGTCATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGTATGCTCGGCAGCG GGGTGTCATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGTATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGTATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGCGGTAAGCATGCTCGGCAGCG GGGTGTGATTGATGGGCGTGGCGGTAGCATTTTGACTGGTGGTAAGCATGCTCGGCAGCG 600
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TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGACCTGGCCTATCAGAACAAGCGCCATGCACTGCATCAACAGGTACCACG TACCTGGTGGGATCTGGCCTATCAGAACAAGCACCATGCACTGCATCAACAGGTACCACG
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GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTTGCCATCGAAAATGC 719 GTTGATCCAAATCAGGGGCGGTAACGACTTCACGTTGTACCGTGTCGCCATCGAAAATGC 720
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599 599 599 599 599 599 599 599 599 599 599 599

659 659 659 659 659 659 659 659 659 659 659 659 660

## (APPENDIX I continued)

XF
$\mathrm{M} 2 \overline{3} \mathrm{~A}-08$
ALSD
Temécula1_PD XF GB514 XF- Oleander XF_A-05
XF B-05
XF_C-05
XF_A-06
$\mathrm{XF}^{-} \mathrm{A}-07$
XF D-07
M1 2 _ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
$\mathrm{XF}^{-} \mathrm{B}-05$
XF_C-05
$\mathrm{XF}^{-} \mathrm{A}-06$
XF_A-07
XF D-07
M1 $\overline{2}$ _ALSD
9a5c_CVC

XF_A-08
M2 $\overline{3}$ ALSD
Temécula1_PD
XF_GB514
$\mathrm{XF}^{-}$- Oleander
XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
XF ${ }^{-}$A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_-Oleander
XF A-05
$\mathrm{XF}-\mathrm{B}-05$
XF_C-05
$\mathrm{XF}^{-}{ }^{-}-06$
XF_A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
M1 $\overline{2}$ _ALSD
9a5c_CVC

ACCTAACTTCCATGTGGTTGCTGATACGGTTAGCGGTGTCACCGCATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTTAGCGGTGTCACCGCATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTTAGCGGTGTCACCGCATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTTAGCGGTGTCACCGCATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGCATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGCGGTGTCACCGTATGGGGCATCCGCAT 779 ACCTAACTTCCATGTGGTTGCTGATACGGTCAGTGGTGTCACCGCATGGGGCATCCGTAT 780


CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCACGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 839 CCTGACCCCAAGCTTGGTCTATACCATGCCGGGGTACCACTGTCCGCCCGGTACCACGCC 840
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GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCCGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 899 GGATGTTGTGACGCCGGCGACATGCTTTACCCCGGACACGGTCAAGAACACCGATGGCTT 900
$\star \star \star \star \star * * * * * * * * * * * * * * * * * * * * * * * * * * * * \quad * * * * * * * * * * * * * * * * * * * * * * * * * *$

TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACCGGGGATGA 959 TGATCCTGGGCAATCAAACCACGTGCTGCTAGCGTACTCCTACATCAGCACTGGGGATGA 960

[^2]
## (APPENDIX I continued)

XF
$\mathrm{M} 2 \overline{3} \mathrm{~A}-08$
ALSD
Temécula1_PD
XF GB514
XF- Oleander
XF_A-05
XF B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
$\mathrm{XF}^{-} \mathrm{A}-07$
XF D-07
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
$X F^{-A}-05$
$X F^{-} B-05$
XF_C-05
$X F^{-} A-06$
XF_A-07
XF D-07
M1 $\overline{2}$ _ALSD
9a5c_CVC

XF_A-08
M2 $\overline{3}$ ALSD
Temecula1_PD
XF_GB514
XF-Oleander
XF_A-05
XF_B-05
XF-C-05
XF_A-06
$X F^{-} A-07$
$\mathrm{XF}^{-} \mathrm{D}-07$
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
$X F-A-05$
XF-B-05
XF_C-05
XF_A-06
XF_A-07
XF $\mathrm{D}-07$
M12_ALSD
9a5c_CVC

CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCTACGCTCTGAGCTTCTTACACAA 1019 CCACGTTGCCATCAAAGCGCGGGGCAAGATGCCAAGCGACGCTCTGAGCTTCTTACACAA 1020

CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACATCGAGTCAGGTGTACACGA 1079 CCACTTTGGCTATGGACATGGCATGTCCATCGGTAGCGACACCGAGTCAGGTGTACACGA 1080
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TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGTTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGTTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGTTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGTTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGTTTTGACAGCCCTAACAGCAATGGCTTGCA 1139 TATGGAAGTCAGTGATTTGAGTATCGACGGCTTTGACAGCCCTAACAGCAATGGCTTGCA 1140

GATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 GATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 GATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 GATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGACCATGTCACCTACTCCAAAATATG 1199 TATGAAATCTGATGCCGATCATGGCGGAGTGGTCGATCATGTCACCTACTCCAAAATATG 1200

## (APPENDIX I continued)

XF
$\mathrm{M} 2 \overline{3} \mathrm{~A}-08$
ALSD
Temécula1_PD
XF GB514
XF- Oleander
XF_A-05
XF B-05
XF_C-05
XF_A-06
$\mathrm{XF}^{-} \mathrm{A}-07$
XF ${ }^{-}$D-07
M12_ALSD
9a5c_CVC

XF A-08
M23_ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
$\mathrm{XF}^{-} \mathrm{B}-05$
XF_C-05
$\mathrm{XF}^{-} \mathrm{A}-06$
XF_A-07
XF D-07
M1 $\overline{2}$ _ALSD
9a5c_CVC

XF_A-08
M2 $\overline{3}$ ALSD
Temécula1_PD
XF_GB514
$\mathrm{XF}^{-}$- Oleander
XF_A-05
XF_B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
XF_A-06
$\mathrm{XF}^{-} \mathrm{A}-07$
$\mathrm{XF}^{-} \mathrm{D}-07$
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
$\mathrm{XF}^{-} \mathrm{A}-05$
$\mathrm{XF}-\mathrm{B}-05$
XF_C-05
$\mathrm{XF}^{-}{ }^{-}-06$
XF_A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
M1 $\overline{2}$ _ALSD
9a5c_CVC

CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1259 CATGCGTCGTCTGAAACGTCCGCTGGCGTTTGACACCTTCTACAAACCTTCCAACGGGAA 1260

TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGT 1319 TTCTTATCCACTCTTCAAAAATATTGTGTTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGCCCTGT 1319 TTCTTATCCACTCTTCAAAAATATCGTGCTGCAAGATATTCATGTGCTTGAAAGTCCTGT 1320
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CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATCTGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTGGGCAGTGGGAATAATATGCCAAT 1379 CTTTGGTGCTGGTCAGCTGCTTTTCATGGGGATCCTCGGCAGTGGGAATAATCTGCCAAT 1380


GACGCTGAGCATGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGCTGAGCATGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGCTGAGCATGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGCTGAGCATGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGCTGAGCATGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGTTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1439 GACGCTGAGTCTGGATAACGTCGTATTCGACGGTTTTTTGCCAACATTGATAGCGCCTCC 1440

[^3]
## (APPENDIX I continued)

XF
$\mathrm{M} 2 \overline{3} \mathrm{~A}-08$
ALSD
Temécula1_PD
XF GB514
XF- Oleander
XF_A-05
XF B-05
$\mathrm{XF}-\mathrm{C}-05$
XF_A-06
$\mathrm{XF}^{-} \mathrm{A}-07$
XF D-07
M12_ALSD
9a5c_CVC

XF A-08
M23_ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF A-05
$\mathrm{XF}-\mathrm{B}-05$
XF_C-05
$\mathrm{XF}^{-} \mathrm{A}-06$
XF_A-07
XF D-07
M1 $\overline{2}$ _ALSD
9a5c_CVC

XF_A-08
M2 $\overline{3}$ ALSD
Teméculal PD
XF_GB514
$\mathrm{XF}^{-}$- Oleander
XF_A-05
XF B-05
$\mathrm{XF}^{-} \mathrm{C}-05$
$\mathrm{XF}_{-}^{-} \mathrm{A}-06$
$\mathrm{XF}^{-} \mathrm{A}-07$
$\mathrm{XF}^{-} \mathrm{D}-07$
M12_ALSD
9a5c_CVC

XF A-08
M2 $\overline{3}$ _ALSD
Temecula1_PD
XF GB514
XF_Oleander
XF-A-05
XF-B-05
XF_C-05
$\mathrm{XF}^{-}{ }^{-}-06$
XF_A-07
$\mathrm{XF}^{-} \mathrm{D}-07$
M1 $\overline{2}$ _ALSD
9a5c_CVC

CAGCAGTGTCGTATTCGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTCGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTCGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTCGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTATCGTATTCGCCAATCCGCAGGCAGCGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTTGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1499 CAGCAGTGTCGTATTCGCCAATCCGCAGGCAGTGCATTTTCACTTCGGTCCCGGTCCGGT 1500


CAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCCCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCTCCTCTGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1559 CAGTTTCGCTCCTTTGATTACCCCCTCGGTTGCTTACGATGTGACTGTGAGTGGAAGTCC 1560
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AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCATATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AAGTGTGGGCAACCCATACGATTGTTCAGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1619 AGGTGTGGGCAACCCATACGATTGTTCGGCGGCATTTATCAATTTTTCCAGCGTATTTCC 1620


TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1635 TGATTCGCCTATCTAA 1636
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(APPENDIX I continued)
ClustalW2 Summary

| SegA | Name | Length | SeqB | Name | Length | Score |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | XF_A-05 | 1635 | 2 | XF_B-05 | 1635 | 100 |
| 1 | XF_A-05 | 1635 | 3 | XF_C-05 | 1635 | 100 |
| 1 | XF_A-05 | 1635 | 4 | XF_A-06 | 1635 | 100 |
| 1 | XF_A-05 | 1635 | 5 | XF_A-07 | 1635 | 100 |
| 1 | XF_A-05 | 1635 | 6 | XF_D-07 | 1635 | 100 |
| 1 | XF_A-05 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 1 | XF_A-05 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 1 | XF_A-05 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 1 | XF_A-05 | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 1 | XF_A-05 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 1 | XF_A-05 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 1 | XF_A-05 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 2 | XF_B-05 | 1635 | 3 | XF_C-05 | 1635 | 100 |
| 2 | XF_B-05 | 1635 | 4 | XF_A-06 | 1635 | 100 |
| 2 | XF_B-05 | 1635 | 5 | XF_A-07 | 1635 | 100 |
| 2 | XF_B-05 | 1635 | 6 | XF_D-07 | 1635 | 100 |
| 2 | XF_B-05 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 2 | XF_B-05 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 2 | XF_B-05 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 2 | XF_B-05 | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 2 | XF_B-05 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 2 | XF_B-05 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 2 | XF_B-05 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 3 | XF_C-05 | 1635 | 4 | XF_A-06 | 1635 | 100 |
| 3 | XF_C-05 | 1635 | 5 | XF_A-07 | 1635 | 100 |
| 3 | XF_C-05 | 1635 | 6 | XF_D-07 | 1635 | 100 |
| 3 | XF_C-05 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 3 | XF_C-05 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 3 | XF_C-05 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 3 | XF_C-05 | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 3 | XF_C-05 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 3 | XF_C-05 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 3 | XF_C-05 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 4 | XF_A-06 | 1635 | 5 | XF_A-07 | 1635 | 100 |
| 4 | XF_A-06 | 1635 | 6 | XF_D-07 | 1635 | 100 |
| 4 | XF_A-06 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 4 | XF_A-06 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 4 | XF_A-06 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 4 | XF_A-06 | 1635 | 10 | Temecula1_PD | 1635 | 98 |

## (APPENDIX I continued)

| SegA | Name | Length | SegB | Name | Length | Score |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 4 | XF_A-06 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 4 | XF_A-06 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 4 | XF_A-06 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 5 | XF_A-07 | 1635 | 6 | XF_D-07 | 1635 | 100 |
| 5 | XF_A-07 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 5 | XF_A-07 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 5 | XF_A-07 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 5 | XF_A-07 | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 5 | XF_A-07 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 5 | XF_A-07 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 5 | XF_A-07 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 6 | XF_D-07 | 1635 | 7 | XF_A-08 | 1635 | 98 |
| 6 | XF_D-07 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 6 | XF_D-07 | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 6 | XF_D-07 | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 6 | XF_D-07 | 1635 | 11 | M12_ALSD | 1635 | 99 |
| 6 | XF_D-07 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 6 | XF_D-07 | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 7 | XF_A-08 | 1635 | 8 | XF_Oleander | 1635 | 98 |
| 7 | XF_A-08 | 1635 | 9 | M23_ALSD | 1635 | 100 |
| 7 | XF_A-08 | 1635 | 10 | Temecula1_PD | 1635 | 100 |
| 7 | XF_A-08 | 1635 | 11 | M12_ALSD | 1635 | 98 |
| 7 | XF_A-08 | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 7 | XF_A-08 | 1635 | 13 | XF_GB514 | 1635 | 100 |
| 8 | XF_Oleander | 1635 | 9 | M23_ALSD | 1635 | 98 |
| 8 | XF_Oleander | 1635 | 10 | Temecula1_PD | 1635 | 98 |
| 8 | XF_Oleander | 1635 | 11 | M12_ALSD | 1635 | 98 |
| 8 | XF_Oleander | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 8 | XF_Oleander | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 9 | M23_ALSD | 1635 | 10 | Temecula1_PD | 1635 | 100 |
| 9 | M23_ALSD | 1635 | 11 | M12_ALSD | 1635 | 98 |
| 9 | M23_ALSD | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 9 | M23_ALSD | 1635 | 13 | XF_GB514 | 1635 | 100 |
| 10 | Temecula1_PD | 1635 | 11 | M12_ALSD | 1635 | 98 |
| 10 | Temecula1_PD | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 10 | Temecula1_PD | 1635 | 13 | XF_GB514 | 1635 | 100 |
| 11 | M12_ALSD | 1635 | 12 | 9a5c_CVC | 1636 | 97 |
| 11 | M12_ALSD | 1635 | 13 | XF_GB514 | 1635 | 98 |
| 12 | 9a5c_CVC | 1636 | 13 | XF_GB514 | 1635 | 97 |

## APPENDIX J: BLAST SEARCH OF PGLA NUCLEOTIDE SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA

| Accession Description |  | Max | Total | Query |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | score | score | coverage | value | ident |
| CP000941.1 | Xylella fastidiosa M12, complete genome | 3014 | 3014 | 100\% | 0.0 | 99\% |
| CP002165.1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | $\underline{2859}$ | 2859 | 100\% | 0.0 | 98\% |
| CP001011.1 | Xylella fastidiosa M23, complete genome | $\underline{2859}$ | 2859 | 100\% | 0.0 | 98\% |
| AE009442.1 | Xylella fastidiosa Temecula1, complete genome | $\underline{2859}$ | 2859 | 100\% | 0.0 | 98\% |
| AE003849.1 | Xylella fastidiosa 9a5c, complete genome | $\underline{2765}$ | 2765 | 100\% | 0.0 | 97\% |

BLAST Query Results: XF A-08 (LA grapevine strain)

| Accession | Description | Max <br> score | $\begin{aligned} & \text { Total } \\ & \hline \text { score } \end{aligned}$ | Query <br> coverage | $E$ <br> value | $\frac{\text { Max }}{\text { ident }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CP002165.1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | 3020 | 3020 | 100\% | 0.0 | 100\% |
| CP001011.1 | Xylella fastidiosa M23, complete genome | 3020 | 3020 | 100\% | 0.0 | 100\% |
| AE009442.1 | Xyiella fastidiosa Temecula1, complete genome | 3020 | 3020 | 100\% | 0.0 | 100\% |
| CP000941.1 | Xylella fastidiosa M12, complete genome | $\underline{2854}$ | 2854 | 100\% | 0.0 | 98\% |
| AE003849.1 | Xylella fastidiosa $9 a 5 \mathrm{c}$, complete genome | 2771 | 2771 | 100\% | 0.0 | 97\% |


| Accession Description |  | Max | Total | Query |  | Max |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | score | score | coverage | value | ident |
| CP002165.1 | Xylella fastidiosa subsp. fastidiosa GB514, complete genome | 2915 | 2915 | 100\% | 0.0 | 98\% |
| CP001011.1 | Xylella fastidiosa M23, complete genome | $\underline{2915}$ | 2915 | 100\% | 0.0 | 98\% |
| AE009442.1 | Xylella fastidiosa Temecula1, complete genome | 2915 | 2915 | 100\% | 0.0 | 98\% |
| CP000941.1 | Xylella fastidiosa M12, complete genome | $\underline{2859}$ | 2859 | 100\% | 0.0 | 98\% |
| AE003849.1 | Xylella fastidiosa 9 a 5 c , complete genome | $\underline{2743}$ | 2743 | 100\% | 0.0 | 96\% |

# APPENDIX K: PGLA AMINO ACID SEQUENCES OF STRAINS OF XYLELLA FASTIDIOSA ISOLATED FROM PLANT HOSTS IN LOUISIANA (TRANSLATED FROM THE PGLA NUCLEOTIDE SEQUENCES) 

>XF_A-05_PGLA

 DANPQASKPDTKRLQDAIDDCPAGSAVKLVIDSHRKSGE L S G P L H L K S R V T L W I D D G V T L F A S R N P K D Y D K G N G T C
 G G K H A R Q R T W W D L A Y Q N K R H A L H Q Q V P R L $\quad$ Q I R G G N D T L Y R V A I E N A P N F H V V A D T V S G V T V W G I R I L T P S L V Y T P GYHCPPGTTPDVVTPATCETPDTVKNTDGEDPGQSNHVLD LA Y S Y I S T G D D H V A I K A R G K Met P S Y A L S L H N H F G Y G H G Met $S$ I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K
 S Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met $G$ I L G S G N L

 S S V F P D S P I Stop
>XF B-05 PGLA
 E P V S V K T L W G EVQR P S L P T H V C T V L P A R L T P K H G S I D A D A N P Q A S K P D T K R L Q D A I D D C P A G S A V K L V I D S H R K G F L S G P L H L K S R V T L W I D D G V T L F A S R N P K D Y D K G N G T C G A T S T H E F S C Met P L I S A I N T T G S G I V G G G V I D G R G G S I T
 L Y R V A I E N A P N F H V V A D T V S G V T V W G I R I L T P S L V Y T P GY H C P P G T T P D V V T P A T C F T P D T V K N T D G F D P G Q S N H V L LA Y S Y I S T G D D H V A I K A R G K Met P S Y A L S F L H N H F G Y G H G Met $S$ I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L Het K S D A D H G GVV D H V T Y S K I C Met R R L K R P L A F D T F Y K P S N G

 G P V S F A P L I T S S V A Y D V T V S G S P S V G N P Y D C S A A F I N S S V F P D S P I Stop
>XF_C-05_PGLA
 E P V S V K T L W G E V Q R P S L P T H V C T V L P AR L T P K H G S I D L
 L S G P L H L K S R V T L W I D D G V T L F A S R N P K D Y D K G N G T C G

 L Y R V A I E N A P N F H V V A D T V S G V T V W G I R I L T P S L V Y T P GYHCPPGTTPDVVTPATCETPDTVKNTDGFDPGQSNHVL LA Y S Y I S T G D D H V A I K A R G K Met P S Y A L S F L H N H F G Y G G Met $S$ I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K S D A D H G G V V D H V T Y S K I C Met R R L K R P L A F D T F Y K P S N C N Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met G I L G G N N L P Met T L S L D N V V F D G F L P T L I A P P S S V V FAN P Q A V H F H F G P
 S V F P D S P I Stop

## (APPENDIX K continued)

```
>XF A-06 PGLA
Met N L D R F L P L V F G L H C F A A Met G G T A DV F R V S Q L S S S D L S
E P V S V K T L W G E V Q R P S L P T H V C T V L P A R L T P K H G S I D A L
D A N P Q A S K P D T K R L Q D A I D D C P A G S A V K L V I D S H R K S G F
L S G P L H L K S R V T L W I D D G V T L F A S R N P K D Y D K G N G T C G T
A T S T H E F S C Met P L I S A I N T T G S G I V G G GV I D G R G G S I L T
GGKHAR Q R T W W D L A Y Q N K R H A L H Q Q V P R L I Q I R G G N D F T
L Y R V A I E N A P N F HV V A D T V S G V T V W G I R I L T P S L V Y T T P
GY H C P P GT T P D V V T P A T C F T P D T V K NT D G F D P G Q S N H V L
L A Y S Y I S T G D D H V A I K A R G K Met P S Y A L S F L H N H F G Y G H G
Met S I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K S
D A D H G G V V D HV T Y S K I C Met R R L K R P L A F D T F Y K P S N G N S
Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met G I L G S G N N L P
Met T L S L D NVV F D G F L P T L I A P P S S V V FAN P Q A V H F H F G P
G P V S F A P L I T S S V A Y D V T V S G S P S V G N P Y D C S A A F I N F S
S V F P D S P I Stop
```

>XF_A-07_PGLA
Met $N \mathrm{~L} D \mathrm{R} F \mathrm{~L} P \mathrm{~L} V \mathrm{~F}$ G L H C F A A Met G G T A D V F R V S Q L S S S L S E P V S V K T L W G E V Q R P S L P T H V C T V L P A R L T P K G S I D A D A N P Q A S K P D T K R L Q D A I D D C P A G S A V K L V I D S H R L S G P L H L K S RVTLW I D D GV T L F A S R N P K D Y D K G N G T C G A T S T H E F S C Met P L I S A I N T T G S G I V G G G V I D G R G G S I T G G K H A R Q R T W W D L A Y Q N K R H A L H Q Q V P R L $\operatorname{L}$ Q I R G G N D T L Y R V A I E N A P N F H V V A D T V S G V T V W G I R I L T P S L V Y T P GY H C P P G T T P D V V T P A T C F T P D T V K N T D G F D P G Q S N H V L LA Y S Y I S T G D D H V A I K A R G K Met P S Y A L S L L H H F G Y G H G Met $S$ I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K S D A D H G G V V D H V T Y S K I C Met R R L K R P L A F D T F Y K P S N C N Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met G I L G S C N L P
 G P V S F A P L I T S S V A Y D V T V S G S P S V G N P Y D C S A A F I N S S V F P D S P I Stop
$>$ XF D-07 PGLA
 E P V S V K T L W G E V Q R P S L P T H V C T V L P A R L T P K G S I D A DANPQASKPDTKRLQDAIDDCPAGSAVKLVIDSHRKSGE L S G P L H L K S R V T L W I D D G V T L F A S R N P K D Y D K G N G T C G T A T S T H E F S C Met P L I S A I N T T G S G I V G G G V I D G R G G S I L G G K H A R Q R T W W D L A Y Q N K R H A L H Q Q V P R L I Q I R G G N D T LYRVAIENAPNEHVVADTVSGVTVWGIRILTPSLVYTTP GY H C P P GT T P D V V T P A T C F T P D T V K N T D G F D P G Q S N H V L LA Y S Y I S T G D D H V A I K A R G K Met P S Y A L S F L H N H F G Y G H G Met $S$ I G S D I E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K S D A D H G G V V D H V T Y S K I C Met R R L K R P L A F D T F Y K P S N N S Y P L F K N I V L Q D I H V L E S P V F G A $\mathcal{C}$ L L F Met G I G S G N N P
 G P V S F A P L I T S S V A Y D V T V S G S P S V G N P Y D C S A A F I N S V F P D S P I Stop

## (APPENDIX K continued)

```
>XF A-08 PGLA
Met N L D R F L P L V F G L H C F A A Met G G T A DV F R V S Q L S S S D L S
E P V S V K T L W G E V Q R P S L P T H V C T V L P A R L T P K H G S I D A L
D A N P K V S K P D T K R L Q D A I D D C P A G S A V K L V I D S H R K S G F
L S G P L H L K S G V T L W I D E G V T L F A S R N P K D Y D K G N G T C G T
A T S T H E F S C Met P L I S A I N T T G S G I V G G GV I D G R G G S I L T
GGKY A R Q R T W W D L A Y Q N K R H A L H Q Q V P R L I Q I R G G N D F T
L Y R V A I E N A P N F H V V A D T V S G V T A W G I R I L T P S L V Y T T P
GY H C P P GT T P D V V T P A T C F T P D T V K N T D G F D P G Q S N H V L
L A Y S Y I S T G D D H V A I K A R G K Met P S Y A L S F L H N H F G Y G H G
Met S I G S D T E S G V H D Met E V S D L S I D G F D S P N S N G L Q Met K S
D A D H G G V V D HV T Y S K I C Met R R L K R P L A F D T F Y K P S N G N S
Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met G I L G S G N N L P
Met T L S Met D N V V F D G F L P T L I A P P S S V V F A N P Q A V H F H F G
P G P V S F A P L I T P S V A Y D V T V S G S P G V G N P Y D C S A A F I N F
S S V F P D S P I Stop
```

>XF_Oleander_PGLA
 E P V S V K T L W G E V Q R P S L P T H V C T V L P A R L T P K G S I D A D A N P K V S K P D T K R L Q D A I D D C P A G S A V K L V I D S H R K S G L S G P L H L K S GV T L W I D D G V T L F A S R N P K D Y D K G N G T C T A T S T H E F S C Met P L I S A I N T T G S G I V G G G V I D G R G G S I T
 L Y R V A I E N A P N F H V V A D T V S G V T A W G I R I L T P S L V Y T P GYHCPPGTTPDVVTPATCETPDTVKNTDGFDPGQSNHVL LAYSYISTGDDHVAIKARGKMet PSYALSELHNHEGYGHG Met $S$ I G S D T E S G V H D Met E V S D L S I D G F D S P N S N G L H Met K S D A D H G G V V D H V T Y S K I C Met R R L K R P L A F D T F Y K P S N C N Y P L F K N I V L Q D I H V L E S P V F G A G Q L L F Met G I G S G N N P
 PGPVSEAPLITPSVAYDVTVSGSPGVGNPYDCSAAFINE S S I F P D S P I Stop

## APPENDIX L: CONSENSUS DATASET OF BANDS PRESENT IN ERIC-PCR AND REP-PCR

Table L1. Consensus Dataset ${ }^{\text {a }}$ of ERIC-PCR Data Used in Phylogenetic Analyses

| $\begin{aligned} & \text { Band } \\ & \text { size }^{\text {b }} \\ & \text { (bp) } \end{aligned}$ | Strain of Xylella fastidiosa |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XF | XF | XF | XF | XF | XF | XF | XF | XF | XF |
|  | A-05 | B-05 | C-05 | A-06 | D-07 | A-10 | B-10 | A-08 | Oleander | A-07 |
| 1,275 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 950 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| 880 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| 800 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 575 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 550 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| 420 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 300 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 160 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| 155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |

${ }^{\text {a }}$ A ' 0 ' indicates the absence of a band in a particular band class, while a ' 1 ' indicates the presence of a band in a particular band class. The dataset was created using distinguishable bands that regularly appeared between 100 bp and $1,650 \mathrm{bp}$ in ERIC-PCR fingerprints.
${ }^{\mathrm{b}}$ Band sizes are approximate sizes based on the location of a band in comparison to the bands of known size present in the DNA marker. Bands present at a particular size make up a 'class'.

Table L2. Consensus Dataset ${ }^{\text {a }}$ of REP-PCR Data Used in Phylogenetic Analyses

| Band | Strain of Xylella fastidiosa |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| size $^{\text {b }}$ | XF | XF | XF | XF | XF | XF | XF | XF | XF | XF |  |
| (bp) | A-05 | B-05 | C-05 | A-06 | D-07 | A-10 | B-10 | A-08 | Oleander | A-07 |  |
| 1,950 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |  |
| 1,900 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |  |
| 1,600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |  |
| 950 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |  |
| 900 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |  |
| 750 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |  |
| 600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |  |
| 520 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |  |
| 460 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |  |

${ }^{\text {a }}$ A ' 0 ' indicates the absence of a band in a particular band class, while a ' 1 ' indicates the presence of a band in a particular band class. The dataset was created using distinguishable bands that regularly appeared between 400 bp and 2,000 bp in REP-PCR fingerprints.
${ }^{\mathrm{b}}$ Band sizes are approximate sizes based on the location of a band in comparison to bands of known size present in the DNA marker. Bands present at a particular size make up a 'class'.

## AWTY Cumulative Plots XF-ITS_cured (stopval=0.01)

XF- ITS_cured (stopval=0.01) Cumulative Plot 1


XF- ITS_cured (stopval=0.01) Cumulative Plot 2


## (APPENDIX M continued)

XF- ITS_cured (stopval=0.01) Cumulative Plot 3
Plot of splits 1 to 20 fron tnp4de88/Cumulative/outm8n $\times 7$ H sorted by uidest range


XF- ITS_cured (stopval=0.01) Cumulative Plot 4


## (APPENDIX M continued)

## AWTY Cumulative Plots XF-PGLA

XF-PGLA Cumulative Plot 1
Plot of splits 1 to 11 fron tnpb9863/Cunulative/outR15gh2 sorted by uidest range


XF-PGLA Cumulative Plot 2


## (APPENDIX M continued)

XF-PGLA Cumulative Plot 3
Plot of splits 1 to 11 fron tnpb9863/Cunulative/outDeS.HC sorted by uidest range


XF-PGLA Cumulative Plot 4
Plot of splits 1 to 11 fron tnpb9863/Cunulative/outRgB4EH sorted by uidest range


## (APPENDIX M continued)

AWTY Cumulative Plots XF-ERIC
XF-ERIC Cumulative Plot 1
Plot of splits 1 to 8 fron tnp1f $45 \mathrm{c} /$ Cunulative/outLolli5 sorted by widest range


XF-ERIC Cumulative Plot 2
Plot of splits 1 to 8 fron tnp1f45c/Cunulative/outRQu6zn sorted by widest range


## (APPENDIX M continued)

XF-ERIC Cumulative Plot 3


XF-ERIC Cumulative Plot 4
Plot of splits 1 to 8 fron tnplf45c/Cunulative/out7thep sorted by widest range


## (APPENDIX M continued)

AWTY Cumulative Plots XF-REP
XF-REP Cumulative Plot 1
Plot of splits 1 to 8 fron tnp39e5e/Cunulative/out7HeifC sorted by uidest range


XF-REP Cumulative Plot 2
Plot of splits 1 to 8 fron tnp39e5e/Cunulative/outS2dsZr sorted by widest range


## (APPENDIX M continued)

XF-REP Cumulative Plot 3
Plot of splits 1 to 8 fron tnp39e5e/Cunulative/out 505 gLh sorted by uidest range


XF-REP Cumulative Plot 4
Plot of splits 1 to 8 fron tnp39e5e/Cunulative/outkTZea8 sorted by uidest range


## (APPENDIX M continued)

## AWTY Cumulative Plots XF-REP-ERIC

XF-REP-ERIC Cumulative Plot 1


XF-REP-ERIC Cumulative Plot 2


## (APPENDIX M continued)

XF-REP-ERIC Cumulative Plot 3
Plot. of splits 1 to 8 fron tmp7d99b/Cumulativefoutmxyela sorten by uidest range


XF-REP-ERIC Cumulative Plot 4
Plot of splits 1 to 8 fron tnp7d93b/Cunulative/outufzOnC sorted by widest range


## (APPENDIX M continued)

AWTY Cumulative Plots XF-IPRE (Total combined dataset)
XF-IPRE Cumulative Plot 1
Plot of splits 1 to 28 fron tnp2459a/Cunulative/outnSHbg4 sorted by uidest range


XF-IPRE Cumulative Plot 2
Plot of splits 1 to 28 fron tnp2459a/Cunulative/outnsHbg4 sorted by uidest range


## (APPENDIX M continued)

XF-IPRE Cumulative Plot 3
Plot of splits 1 to 28 fron tnp $2459 \mathrm{a} /$ Cunulative/outnshbg 4 sorted by uidest range


XF-IPRE Cumulative Plot 4
Plot of splits 1 to 20 fron tnp2459a/Cunulative/outr80zp8 sorted by widest range


## (APPENDIX M continued)

## AWTY Compare Plots XF-ITS_cured (stopval=0.01)



## (APPENDIX M continued)

## AWTY Compare Plots PGLA








## (APPENDIX M continued)

## AWTY Compare Plots ERIC-PCR



## (APPENDIX M continued)

## AWTY Compare Plots REP-PCR



## (APPENDIX M continued)

## AWTY Compare Plots ERIC-PCR and REP-PCR Combined Dataset



## (APPENDIX M continued)

## AWTY Compare Plots Total Combined Dataset








## APPENDIX N: PROCEDURE FOR SEQUENCING GENES USED IN A MULTILOCUS SEQUENCE TYPING SYSTEM FOR XYLELLA FASTIDIOSA

Primers for six different housekeeping genes (Table N1) used in a multilocus sequence typing (MLST) system (Schuenzel et al., 2005) were used to amplify the partial sequences of these genes from five strains of X. fastidiosa, XF A-05, XF B-05, XF C-05, XF A-07, and XF A08 (Table 1). The PCR cycling parameters and procedure, modified from Schuenzel et al., (2005), are described in Table N2.

Table N1. Multilocus Sequence Typing Genes and Primers Used for Gene Amplification

| Gene ID | Gene length (bp) | Primer name ${ }^{\text {a }}$ | Primer sequence ( $\left.5^{\prime} \rightarrow 3^{\prime}\right)^{\text {b }}$ |
| :---: | :---: | :---: | :---: |
| cysG | 1,170 | cysG_F | GGCGGCGGTAAGGTTG |
|  |  | cysG_R | GCGTATGTCTGTGCGGTGTGC |
| $g l t T$ | 951 | gltT_F | TTGGGTGTGGGTACGTTGCTG |
|  |  | gltT_R | CGCTGCCTCGTAAACCGTTGT |
| holC | 342 | holC_F | GATTTCCAAACCGCGCTTTC |
|  |  | holC_R | TCATGTGCAGGCCGCGTCTCT |
| petC | 531 | petC_F | CTGCCATTCGTTGAAGTACCT |
|  |  | petC_R | CGTCCTCCCAATAAGCCT |
| pilU | 873 | pilU_F | CAATGAAGATTCACGGCAATA |
|  |  | pilU_R | ATAGTTAATGGCTCCGCTATG |
| $r f b D$ | 429 | rfbD_F | TTTGGTGATTGAGCCGAGGGT |
|  |  | rfbD_R | CCATAAACGGCCGCTTTC |

${ }^{\mathrm{a}}$ Primer names were assigned for the purposes of ordering.
${ }^{\mathrm{b}}$ Primer sequences were obtained from Schuenzel et al., (2005).

Aliquots of the PCR products were tested by gel electrophoresis as described in Table N2 to confirm PCR product. The remaining PCR products were purified using the QuickClean 5M PCR Purification Kit (GenScript, Piscataway, NJ). Purified PCR products were sequenced by Macrogen Inc., Seoul, Korea.

MLST gene sequences were analyzed with Geneious Pro 5.4 (Biomatters LTD, Auckland, New Zealand) (Drummond et al., 2011). When possible, sequences from two independent PCRs for each gene for each strain were aligned to ensure correct base

## (APPENDIX N continued)

identification. When only a single readable sequence was returned, the $\mathrm{HQ} \% \mathrm{DNA}$ for the edited sequence was recorded (Appendix O).

Table N2. PCR and Electrophoresis Conditions to Amplify and Visualize Multilocus Sequence Typing Gene Sequences and PCR Products

| Genes amplified | PCR reaction components ${ }^{\text {s }}$ | PCR program ${ }^{\text {b }}$ | $\begin{gathered} \text { Gel } \\ \text { electrophoresis }^{\mathrm{c}} \end{gathered}$ |
| :---: | :---: | :---: | :---: |
| cysG, glt , | $50 \mu \mathrm{~L}$ volume: $2 \mu \mathrm{l}$ of template | $94^{\circ} \mathrm{C}$ for 3 min ; | 0.7 to 1.0\% |
| holC, petC, | DNA, $5.0 \mu \mathrm{l}$ of 10X PCR | 20 cycles: $94^{\circ} \mathrm{C}$ for 30 s , | agarose gel |
| pilU, rfbD | Buffer, $2.0 \mu$ each of the appropriate primers, $1.5 \mu \mathrm{l}$ of | $60^{\circ} \mathrm{C}$ for $30 \mathrm{~s}, 72^{\circ} \mathrm{C}$ for 1 $\min$ for products $<1000$ |  |
|  | $\mathrm{MgCl}_{2}, 1.0 \mu \mathrm{l}$ of dNTP mix, | bp or 90 s for $>1000 \mathrm{bp}$; |  |
|  | $1.5 \mu \mathrm{l}$ of Taq polymerase | $72^{\circ} \mathrm{C}$ for 5 min |  |

${ }^{\mathrm{a}}$ PCR reagents and DNA template solutions were used at the following concentrations: 10X PCR Buffer; homemade Taq polymerase, $\sim 1.0 \mathrm{U} / \mu \mathrm{l}$; primers, $10 \mu \mathrm{M} ; \mathrm{MgCl}_{2}, 50 \mathrm{mM}$; dNTP mix, 10 mM ; DNA template, $1 \mathrm{ng} / \mu$ l. Sterile $\mathrm{ddH}_{2} \mathrm{O}$ was used to bring the volume up to the desired reaction volume.
${ }^{\mathrm{b}}$ PCR was performed in a DNA Engine Peltier Thermal Cycler (Bio-Rad, Hercules, CA). Program was modified from Schuenzel et al., (2005).
${ }^{\mathrm{c}}$ Agarose gels contained ethidium bromide for visualization of PCR products under UV light. PCR products were visualized and photographed with a KODAK Gel Logic 1500 Imaging System (Eastman Kodak Company, Rochester, New York). Unless specified, gels were run under standard conditions in 1X Tris-borate EDTA (TBE) buffer (Sambrook and Russell, 2001).

## References

Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. and Wilson, A. 2011. Geneious v5.4, available from http://www.geneious.com/.

Sambrook, J. and Russell, D. W. Molecular cloning: A laboratory manual. 3rd edition. Cold Spring Harbor Laboratory Press. 2001. 2344 pages.

Schuenzel, E. L., Scally, M., Stouthamer, R. and Nunney, L. 2005. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen Xylella fastidiosa. Applied and Environmental Microbiology 71:3832-3839.

## APPENDIX O: NUCLEOTIDE SEQUENCES OF GENES USED IN THE MULTILOCUS SEQUENCE TYPING ANALYSIS FOR XYLELLA FASTIDIOSA FROM STRAINS OF XYLELLA FASTIDIOSA FROM LOUISIANA

Gene Nucleotide Sequence Summary

| Strain designation | cys $G^{\text {a }}$ |  | glt $T^{\text {a }}$ |  | holC ${ }^{\text {a }}$ |  | petC ${ }^{\text {a }}$ |  | pilU ${ }^{\text {b }}$ |  | $r f b D^{\text {a }}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{gathered} \mathbf{H Q} \\ \% \end{gathered}$ | Size <br> (bp) | $\begin{gathered} \mathrm{HQ} \\ \% \end{gathered}$ | Size <br> (bp) | $\begin{gathered} \mathrm{HQ} \\ \% \end{gathered}$ | Size (bp) | $\begin{gathered} \mathrm{HQ} \\ \% \end{gathered}$ | Size (bp) | $\begin{gathered} \mathrm{HQ} \\ \% \\ \hline \end{gathered}$ | Size (bp) | $\begin{gathered} \text { HQ } \\ \% \\ \hline \end{gathered}$ | Size (bp) |
| XF A-05 | 92.0 | 697 | 59.7 | 698 | 98.4 | 313 | 84.8 | 492 | -- | 839 | 69.9 | 412 |
| XF B-05 | 68.5 | 588 | 88.6 | 623 | 98.1 | 311 | 74.8 | 480 | -- | 752 | 34.3 | 426 |
| XF C-05 | 52.7 | 412 | 53.3 | 499 | 92.5 | 306 | -- | -- | -- | 532 | 40.1 | 419 |
| XF A-07 | 84.5 | 503 | 63.6 | 503 | 67.1 | 286 | 49.0 | 480 | -- | 546 | -- | -- |
| XF A-08 | 97.5 | 674 | 82.9 | 702 | 96.4 | 308 | 96.1 | 490 | -- | 753 | 46.9 | 397 |

${ }^{\text {a }}$ Sequences obtained from a single independent PCR product.
${ }^{\mathrm{b}}$ Two independent PCR products were used to obtain sequences for pilU.

## Nucleotide Sequences

## cys $G$

>XF_A-05_cysG
GATTATCGGCGCCCCCTCTCTGACCACATCGTTACAGCGCTGGGCCGAAACCGGCCGCATCACG TGGCGGCAAGGGACGTTTGAAGACAGCTGGCTGCAAGAAGATATTTGGTTGGTCATCGCCGCCA CCGATCAACCCGAAGTGAACCACGCCGCGGCGCGTGCAGCACATGCGCAACGGTTATTCGTCAA CGTGGTCGACGACATTGCACTCTCCAACGTGCAGGTCCCGGCCGTGGTCGAACGCGGCCCGTTG CGGATCGCGATTTCTAGCGGCGGTGGCGCACCGATGGTGGCACGCTATCTGCGACAACAACTGG AAAGCCTCATTGACGATAGTTGGGGACGACTGACCACACTGTTCGCGCAACGCCGCGACACTAT CCGCGCCCGCTATCCAAACATAGAAGCACGCCGCCGCTTCTTTGAAACCCAACTCGCCGGCCCA CTCCAACGACTGCTACGCAAGCAACGCCACGCAGAGGCCGAAGCAGTGCTGGAAGCAGCACTCG CCGAGACTCCCCTCACGGAGTCCGGCAGCGTCACCCTAGTGGGCGCTGGCGCCGGCGACGCCGG GCTACTGACCTTAAATGCACTGCGCGCCTTGAATGAAGCCGACATCATCCTCTACGACCGCCTG GTCAGCGACACCGTGTTACAGATGGCACGGCGCGATGCGGAGCAGATCGAAGTAGGC
>XF_B-05_cysG
GCCCCCTCTCTGACCACATCGTTACAGCGCTGGGCCGAAACCGGCCGCATCACGTGGCGGCAAG GGACGTTTGAAGACAGCTGGCTGCAAGAAGATATTTGGTTGGTCATCGCCGCCACCGATCAACC CGAAGTGAACCACGCCGCGGCGCGTGCAGCACATGCGCAACGGTTATTCGTCAACGTGGTCGAC GACATTGCACTCTCCAACGTGCAGGTCCCGGCCGTGGTCGAACGCGGCCCGTTGCGGATCGCGA TTTCTAGCGGCGGTGGCGCACCGATGGTGGCACGCTATCTGCGACAACAACTGGAAAGCCTCAT TGACGATAGTTGGGGACGACTGACCACACTGTTCGCGCAACGCCGCGACACTATCCGCGCCCGC TATCCAAACATAGAAGCACGCCGCCGCTTCTTTGAAACCCAACTCGCCGGCCCACTCCAACGAC TGCTACGCAAGCAACGCCACGCAGAGGCCGAAGCAGTGCTGGAAGCAGCACTCGCCGAGACTCC CCTCACGGAGTCCGGCAGCGTCACCCTAGTGGGCGCTGGCGCCGGCGACGCCGGGCTACTGACC TTAAATGCACTG
>XF_C-05_cysG
GCCGCGGCGCGTGCAGCACATGCGCAACGGTTATTCGTCAACGTGGTCGACGACATTGCACTCT CCAACGTGCAGGTCCCGGCCGTGGTCGAACGCGGCCCGTTGCGGATCGCGATTTCTAGCGGCGG TGGCGCACCGATGGTGGCACGCTATCTGCGACAACAACTGGAAAGCCTCATTGACGATAGTTGG GGACGACTGACCACACTGTTCGCGCAACGCCGCGACACTATCCGCGCCCGCTATCCAAACATAG

## (APPENDIX O continued)

AAGCACGCCGCCGCTTCTTTGAAACCCAACTCGCCGGCCCACTCCAACGACTGCTACGCAAGCA ACGCCACGCAGAGGCCGAAGCAGTGCTGGAAGCAGCACTCGCCGAGACTCCCCTCACGGAGTCC GGCAGCGTCACCCTAGTGGGCGCTGGCG

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>XF_A-07_cysG
```

AAACCGGCCGCATCACGTGGCGGCAAGGGACGTTTGAAGACAGCTGGCTGCAAGAAGATATTTG GTTGGTCATCGCCGCCACCGATCAACCCGAAGTGAACCACGCCGCGGCGCGTGCAGCACATGCG CAACGGTTATTCGTCAACGTGGTCGACGACATTGCACTCTCCAACGTGCAGGTCCCGGCCGTGG TCGAACGCGGCCCGTTGCGGATCGCGATTTCTAGCGGCGGTGGCGCACCGATGGTGGCACGCTA TCTGCGACAACAACTGGAAAGCCTCATTGACGATAGTTGGGGACGACTGACCACACTGTTCGCG CAACGCCGCGACACTATCCGCGCCCGCTATCCAAACATAGAAGCACGCCGCCGCTTCTTTGAAA CCCAACTCGCCGGCCCACTCCAACGACTGCTACGCAAGCAACGCCACGCAGAGGCCGAAGCAGT GCTGGAAGCAGCACTCGCCGAGACTCCCCTCACGGAGTCCGGCAGCGTCACCCTA

## >XF_A-08_cysG

GCCCCCTCTCTGACCGCATCGTTACAGCGCTGGGCCGAAACCGGTCGCATCACGTGGCGGCAAG GGACGTTTGAAAACAGCTGGCTGCAAGAAGATATTTGGTTGGTCATCGCCGCCACCGATCAACC CGAGGTGAACCACGCCGCGGCGCGCGCAGCACATGCGCAACGGTTATTCGTCAACGTGGTCGAC GACATTGCACTCTCCAACGTGCAGGTCCCGGCCGTGGTCGAACGCGGCCCGTTGCGGATCGCGA TTTCTAGCGGCGGTGGCGCACCGATGGTGGCACGCTATCTGCGACAACAACTGGAAAGCCTCAT TGACGATAGTTGGGGACGACTGACCACACTGTTCGCGCAACGCCGCGACACTATCCGCGCCCGC TATCCAAACATAGAAGCACGCCGCCGCTTCTTTGAAACCCAACTCGCCGGCCCACTCCAACGAT TGCTACGCAAGCAACGCCACGCAGAGGCCGAAGCAGTGCTGGAAGCAGCACTCGCCAAGACTCC CCCCACGGAGTCCGGCAGCGTCACCCTGGTGGGCGCTGGCGCCGGCGACGCCGGGCTACTGACC TTAAATGCACTGCGCGCCTTGAATGAAGCCGACATCATCCTCTACGACCGCCTGGTCAGCGACA CCGTGTTACAGATGGCACGGCGCGATGCGGAGCA

## gltT

>XF_A-05_gltT
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>XF_B-05_gltT
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## (APPENDIX O continued)

GGAGGTACGCAAGCTGATCGGGCAGATGAGCGAGATCATGATCCAAATCACTCGCTTTGTGCTG GAGATGACGCCACTGGGGACCTTTGGATTAATCGCTGCGTTAGTCGGTAGCTATGGTTTTCAGA AGTTGCTGCCTTTCGGTCACTTTGTATTGGCACTTTATTTGGCATGTACGTTGCACATTGTGGT GGTGTACAGCGGGCTTCTGCTGGCACATGGTTTGAGTCCATTGAAGTTTTTCAGGGGTGTCGCG CCAGGGATGCAGGTCGCTTTTGTCAGTTCTTCCAGCTTTGCTGCGATGCCAGTGGCGTTACGCG CGATTACGCACAATCTGGGGGTTAACAAGGACTACGCTGCATTTGCTGTGCCGCTGGGTAGTAG CATCAAGATGGACGGTTGCGGGGCGATCTTTCCAGCGTTGTGTGCGG
>XF_C-05_gltT
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>XF_A-07_gltT
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>XF_A-08_gltT
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## holC

>XF_A-05_holC
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## (APPENDIX O continued)

AGACGACGACATCACCCCCGTATTGATTGCCACCCCGGACAGCGACACCCCATCGCGCCCGCTG GTCATCAACCTACGCGACGCCCCTTGGGACGGCCCCTGCGAACGCGTGTTGGAGGTGGTTCCCG CCGATCCTGCGGCACGCGAGCCACTGCGTGAACGCTGGAAACACTACAAGAGACGCG
>XF_B-05_holC
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>XF_C-05_holC
ACACCCATCAGCCCGCGCTGATTCTGGCCCGCGACCAAGCCCAGGCCGAAGCACTGGATGACCT ACTGTGGGCGTTCGACCCGGACGCCTACATCCCCCACCAAATCGCCGGCAGCGACGAAGACGAC GACATCACCCCCGTATTGATTGCCACCCCGGACAGCGACACCCCATCGCGCCCGCTGGTCATCA ACCTACGCGACGCCCCTTGGGACGGCCCCTGCGAACGCGTGTTGGAGGTGGTTCCCGCCGATCC TGCGGCACGCGAGCCACTGCGTGAACGCTGGAAACACTACAAGAGACGCG
>XF_A-07_holC
GCCCGCGCTGATTCTGGCCCGCGACCAAGCCCAGGCCGAAGCACTGGATGACCTACTGTGGGCG TTCGACCCGGACGCCTACATCCCCCACCAAATCGCCGGCAGCGACGAAGACGACGACATCACCC CCGTATTGATTGCCACCCCGGACAGCGACACCCCATCGCGCCCGCTGGTCATCAACCTACGCGA CGCCCCTTGGGACGGCCCCTGCGAACGCGTGTTGGAGGTGGTTCCCGCCGATCCTGCGGCACGC GAGCCACTGCGTGAACGCTGGAAACACTAC

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>XF_A-08_holC
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CTACGACACCCATCAGCCCACACTGATTCTGGCCCGCGACCAGGCCCAGGCCGAAGCACTGGAT GACCTACTGTGGGCGTTCGACCCGGACGCCTACATCCCCCACCAAATCGCTGGCAGCGACGAAG ACGACGACATCACCCCCGTATTGATTGCCACCCCGGACAGCGACACCCCATCGCGCCCGCTGGT CATCAACCTACGCGACGCCCCTTGGGATGGCCCCTGCGAACGCGTGTTGGAGGTGGTTCCCGCT GATCCTGCGGCACGCGAGCCACTGCGTGAACGCTGGAAACACTACAAGAGAC

## petC

>XF_A-05_petC
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>XF_B-05_petC
GAGTACTCTCAATGTCACCGGTGCCAAGTTTGGCGATACGATCATGACAGGGATGCCGGTTGAC

## (APPENDIX O continued)

ACTTCGGAGCAGTGGTTTGGTAAGATTCCGCCGGATTTGAGTTTGGTGGCCCGCGTCCGTGGGA GTGATTGGATCTACACTTATCTGAGGTCTTTCTACGTCGACTCGACTCGCCCGCTGGGTTGGAA CAATCGACTGTTTGTCAATGTTTCTATGCCCAATCCACTGTCGCATCTCCAGGGTGTGCAGCGG GCAAAATATGGTGGGGCGTCGCAGGCTGGTGCTGATCGGTTAGTGACTGGGTTGGTTCTGGTCC AGCCAGGTCAGCAGAACCCTGCTGAGTTTGACCAGACTCTGCGTGACATTGTCAATTTCCTGCA ATATGCCGCCGAGCCTGCGGCGTTACAACGGCATTCTTTGAGAGTATGGGTACTGTTGTTCCTA GTGTTGTTGACTTTCTTGGTTTATCTACTTAA
>XF_A-07_petC
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>XF_A-08_petC
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## pilU

>XF_A-05_pilU
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## (APPENDIX O continued)

>XF_B-05_pilU

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## >XF_C-05_pilU

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>XF_A-07_pilU
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## >XF_A-08_pilU

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## (APPENDIX O continued)

CCGGCCATCTGGTCATGTGTACCCTACATGCCAACAACGCCAATCAAGCAATAGACCGCATTAT TAACTTCTTCCCGGAAGAGCGGCGCAGCCAATTACTGATGGATCTGTCGCTGAATCTAAAAGGC ATCATCGCTCAACAATTGATCCCATCTGCGGATGGTAAAGAGCGCTGTTTAGCAGCGGAAGTTT TGCGCGACAGTCGATTAGTACAGGACTACATACGCGTAGGCGAGATCCA

## $r f b D$

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## VITA

Rebecca A. Melanson grew up in Marrero, Louisiana, where she attended Concordia Lutheran School and Immaculata High School. She attended Centenary College of Louisiana in Shreveport, Louisiana, from 1999 to 2003 and earned a Bachelor of Science in biology with minors in chemistry and psychology in 2003. Following graduation, she worked as an entomology student worker at the Louisiana State University (LSU) Agricultural Center (LSU AgCenter) Red River Research Station in Bossier City, Louisiana. In January 2004, she became the research associate in plant pathology at the LSU AgCenter Pecan Research-Extension Station in Shreveport, Louisiana, where she worked until August 2008. During her time as a research associate, she conducted research with Dr. R. S. Sanderlin on the transmission of Xylella fastidiosa, the causal agent of pecan bacterial leaf scorch, in pecan, as well as on pecan scab disease. She was also the Content Management System's Content Reviewer for the LSU AgCenter's Pecan Research-Extension Station website and, eventually, for the LSU AgCenter's Northwest Region. In August 2008, she joined Louisiana State University's Department of Plant Pathology and Crop Physiology as a graduate student under Dr. Jong Hyun Ham where she continued working with Xylella fastidiosa and began characterizing the function of a negative regulator for toxoflavin production in Burkholderia glumae, the causal agent of bacterial panicle blight in rice. As a graduate student, she served as the editor of the Department of Plant Pathology and Crop Physiology newsletter and as the president of the Department of Plant Pathology and Crop Physiology Graduate Student Association. She received travel awards from the LSU Graduate School, the Department of Plant Pathology and Crop Physiology Graduate Student Association, and the American Phytopathological Society - Southern Division to attend American Phytopathological Society's national and regional meetings in 2009, 2010, and 2011.

She also received the 2010 Louisiana Agricultural Consultants Association Scholarship. Rebecca will continue her graduate studies in the Department of Plant Pathology and Crop Physiology at LSU under Dr. Jong Hyun Ham.


[^0]:    ${ }^{1}$ A manuscript containing a major portion of the following chapter, the thesis abstract, and Appendix B, coauthored by R. S. Sanderlin, A. R. McTaggart, and J. H. Ham, has been submitted for publication to Plant Disease under the title, "A systematic study of the 16S-23S rRNA internal transcribed spacer region, pglA, and ERIC-PCR and REP-PCR fingerprints reveals that Xylella fastidiosa strains from pecan are part of $X$. fastidiosa subsp. multiplex" (Appendix A).

[^1]:    *********** ** ******************************************

[^2]:    

[^3]:    **** **** *****************************************************

