



Distribution and genetic analysis of *Xylella fastidiosa* strains found in chitalpa in the southwest United States.

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Abstract

Chitalpa is a common landscape plant used in the desert southwest United States. In the summer of 2006, *Xylella fastidiosa*, a xylem-limited bacterium known to cause disease in many different plants, was detected in chitalpa trees. At the same time, *X. fastidiosa* was detected for the first time in grapes grown in New Mexico. The common use of chitalpa as a landscape plant coupled with the recent discovery that it can harbor *X. fastidiosa* prompted us to survey chitalpa trees across the southwest. Leaves from established chitalpa trees exhibiting symptoms of leaf scorch and dieback were collected from New Mexico and Arizona. Samples were also collected from nursery stock imported into New Mexico from California. These samples were evaluated for the presence of *X. fastidiosa* by ELISA, PCR, and culturing. The results of this survey show that chitalpa trees from New Mexico, Arizona, and California are frequently infected with *X. fastidiosa*. Initial sequence based phylogenetic analysis suggests a close relationship between the *X. fastidiosa* strains associated with the first known occurrence of Pierce's disease in New Mexico. Current research is being conducted to determine if *X. fastidiosa* causes disease in chitalpa and to what extent the chitalpa isolate of *X. fastidiosa* will infect other hosts.

INTRODUCTION

Xylella fastidiosa is a gram negative bacterium that resides within the xylem and causes serious disease problems in many diverse plant species. *X. fastidiosa* is transmitted by xylem feeding insect vectors such as sharpshooters, leafhoppers, and spittle bugs [18]. Diseases caused by *X. fastidiosa* include Pierce's disease in grapes [6], citrus variegated chlorosis (CVC) [5], coffee leaf scorch [11], pecan leaf scorch [19], phony peach [22], plum leaf scald [16], and almond leaf scorch [11]. *X. fastidiosa* has also been shown to be the causative agent of diseases found in landscape plants such as oleander leaf scorch [15], mulberry leaf scorch [8], and oak leaf scorch [3]. In addition to the examples above proven through the completion of Koch's postulates *X. fastidiosa* is known to be associated with several other ornamental landscape species including crape myrtle, olive, day lily, and Southern magnolia [9].

In the desert southwest region of the United States finding suitable landscape plants which can survive the harsh semi-arid conditions can be a challenge. Chitalpa (*Chitalpa tashkentensis* Elias and Wisura) is an ornamental landscape plant that was developed for such arid conditions. It has been utilized in California, Arizona, and New Mexico and is heavily planted in some areas such as Southern New Mexico. Chitalpa was originally bred in Russia and introduced into the United States in 1977 and is an intergenic hybrid between desert willow (*Chilopsis linearis* Cav.) and *Catalpa bignonioides* Walt. [12]. In the past, chitalpa trees across the Southwest have been observed to display leaf scorch symptoms of unknown origin. In the summer and fall of 2006, many chitalpa trees in southern New Mexico and Arizona exhibited leaf scorch. We recently reported that *X. fastidiosa* was detected in chitalpa trees that displayed leaf scorch symptoms in southern New Mexico. We also recently reported the first known occurrence of Pierces disease in New Mexico and noted that the strains of *X. fastidiosa* found in infected New Mexico grapes were highly similar to those present in chitalpa trees from the same area. The common use of chitalpa as a landscape plant in the southwest coupled with the recent discovery that it can harbor *X. fastidiosa* strains similar to those causing Pierce's disease in New Mexico prompted us to survey chitalpa trees across the southwest.

Methods and Materials

Collection of chitalpa and oleander samples. Samples were taken from chitalpa trees exhibiting leaf scorch type symptoms from Southern New Mexico during the summer and fall of 2006. Chitalpa samples were also collected from Tucson and Sierra Vista Arizona and commercial nurseries in Southern New Mexico in October of 2006. Samples from these plants consisted of stems, leaves, and branches.

ELISA of symptomatic chitalpa plants. The presence of *X. fastidiosa* was first tested for by enzyme-linked immunosorbent assay (ELISA). Two different methods were utilized for this assay, first, 0.5 grams to 1.0g of leaf petioles and the mid-veins were placed in plastic samples bags with 3 ml to 5 ml of extraction buffer 3 (Agdia, Inc. Elkhart IN) and the tissue was crushed with the use of a hammer at room temperature. Second, the sap was extracted from chitalpa branches using a pressure chamber (Soilmoisture Equipment, Santa Barbara CA) pressurized with compressed nitrogen gas. Sap was obtained between 20 and 40 bars of pressure. These crushed samples and extracted sap were then loaded into strips coated with *X. fastidiosa* specific antibodies (X.f. PathoScreen Kit, AgDia, Inc.) and processed as per the manufacturer's instructions (Agdia Inc.). Results were analyzed for the presence of color and using a plate reader (Bio-Tek KC4 v.3.1) at 620 nm. All test plates included at least three negative controls and samples were considered positive at two times the background of the negative control.

Bacterial plating from chitalpa leaf tissue. Leaves were surface sterilized by submerging in 70% ethanol for two minutes followed by submerging the leaf in 30% bleach (1.5% sodium hypochlorite) for two minutes. The leaves were rinsed in sterile distilled water twice. Leaf sections, consisting of mainly the petiole and main veins, were finely chopped on sterile filter paper and placed in an eppendorf tube with 600 microliters of sterile succinate-citrate-phosphate buffer. Leaf pieces were ground using a homogenizer for 30 seconds. Ten microliters of this extract was added to 90 microliters of sterile succinate-citrate-phosphate buffer and plated on XfD2 media. The plates were incubated at 28°C and monitored for colony development for five weeks.

Total DNA extraction and PCR. Total DNA was extracted from chitalpa plant samples using the Qiagen Plant DNAeasy kit (Qiagen Inc, Valencia, CA). The quality of the DNA was verified on a 1% agarose gel and by PCR amplification of a segment of the actin gene as an internal control. Actin amplification was performed using actin gene specific primers, actin A:GGACTCTGGAGATGGTG and actin B:GCAGCTTCCATTCCGATC. The components to the PCR reaction included 1X PCR Buffer (100mM Tris-HCl, 500mM KCl, pH 8.3), 1.5mM MgCl₂, 0.2mM dNTP's, 0.1 ng of each primer, two units of Taq Polymerase and 1ul total chitalpa DNA. The reaction conditions were as follows: an initial denaturation step of 95°C for 2 minutes, thirty cycles of the following: 95°C for 45 seconds, 51°C for 45 seconds, and 72°C for 2 minutes, with a final elongation step of 72°C for 5 minutes. The 350 base pair actin band was then visualized on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light with the Kodak Image 2000R Station (Eastman Kodak Company, Rochester, NY). PCR detection of *X. fastidiosa* with total DNA, xylem fluid and bacterial colonies. Total DNA isolated from chitalpa plants or expressed xylem fluid (diluted 1:100) obtained from the pressure chamber (see ELISA methods) was used for polymerase chain reaction (PCR) analysis. The 272-1 and 272-2 external and internal primers for nested PCR were utilized to determine the presence of *X. fastidiosa* as previously described by Pooler et al. [13]. The PCR components for these reactions were the same as described for actin above. Templates consisted of one microliter of the total chitalpa DNA, one microliter of 1:100 dilution of xylem fluid or a "touch" of the bacterial colony for whole cell PCR. The reaction conditions were as follows: an initial denaturation step of 95°C for 2 minutes, thirty-five cycles of the following: 95°C for 45 seconds, 55°C for 45 seconds, and 72°C for 2 minutes, with a final elongation step of 72°C for 5 minutes. The products were then separated on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light with the Kodak 2000R Station (Eastman Kodak Company, Rochester, NY).

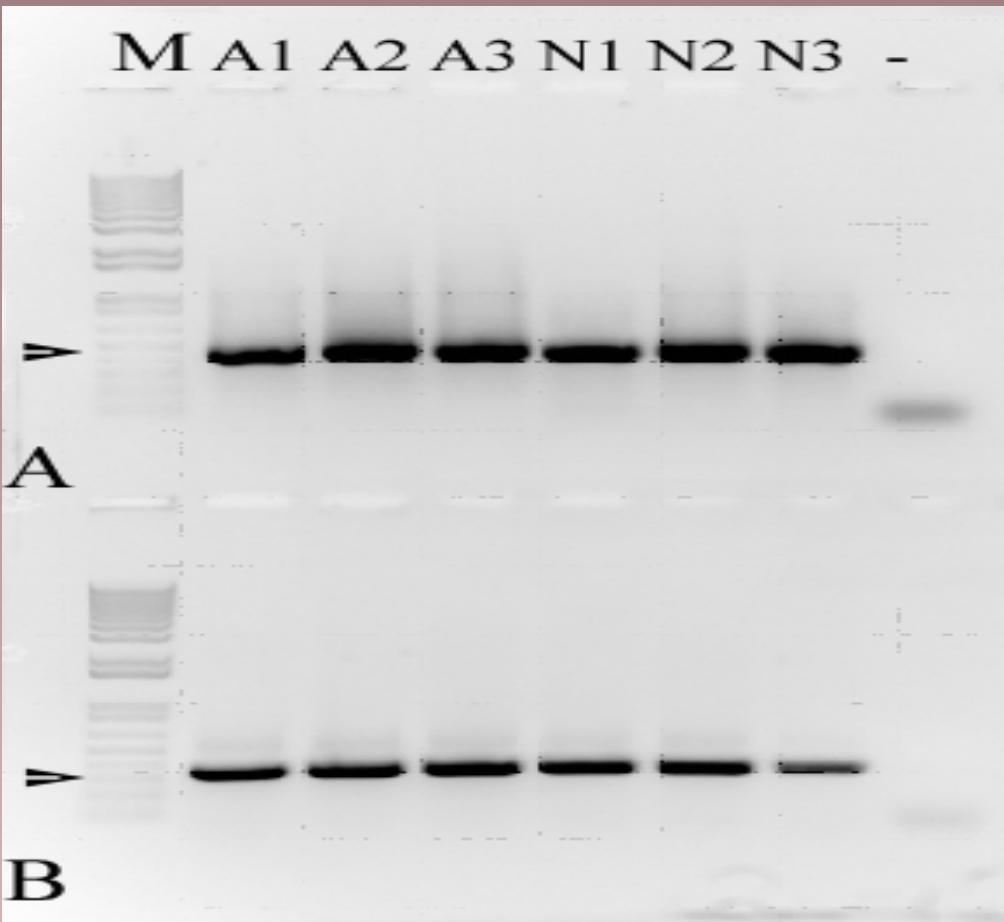
DNA sequencing and sequence analysis. The PCR products were directly sequenced using Big Dye Terminator version 3.1 kit (ABI, Foster City, CA). Sequencing reactions were purified using Performa DTR gel filtration cartridges (Edge Bio System, Gaithersburg, MD)and run on an ABI3100 automated sequencer (NMSU-LiCor facility). The sequences were analyzed using the sequence scanner software (BioRad, Hercules, CA). Sequences were blasted using the NCBI website. Sequences were aligned and phylogenetic relationships were determined using Geneious Pro 2.5.3.



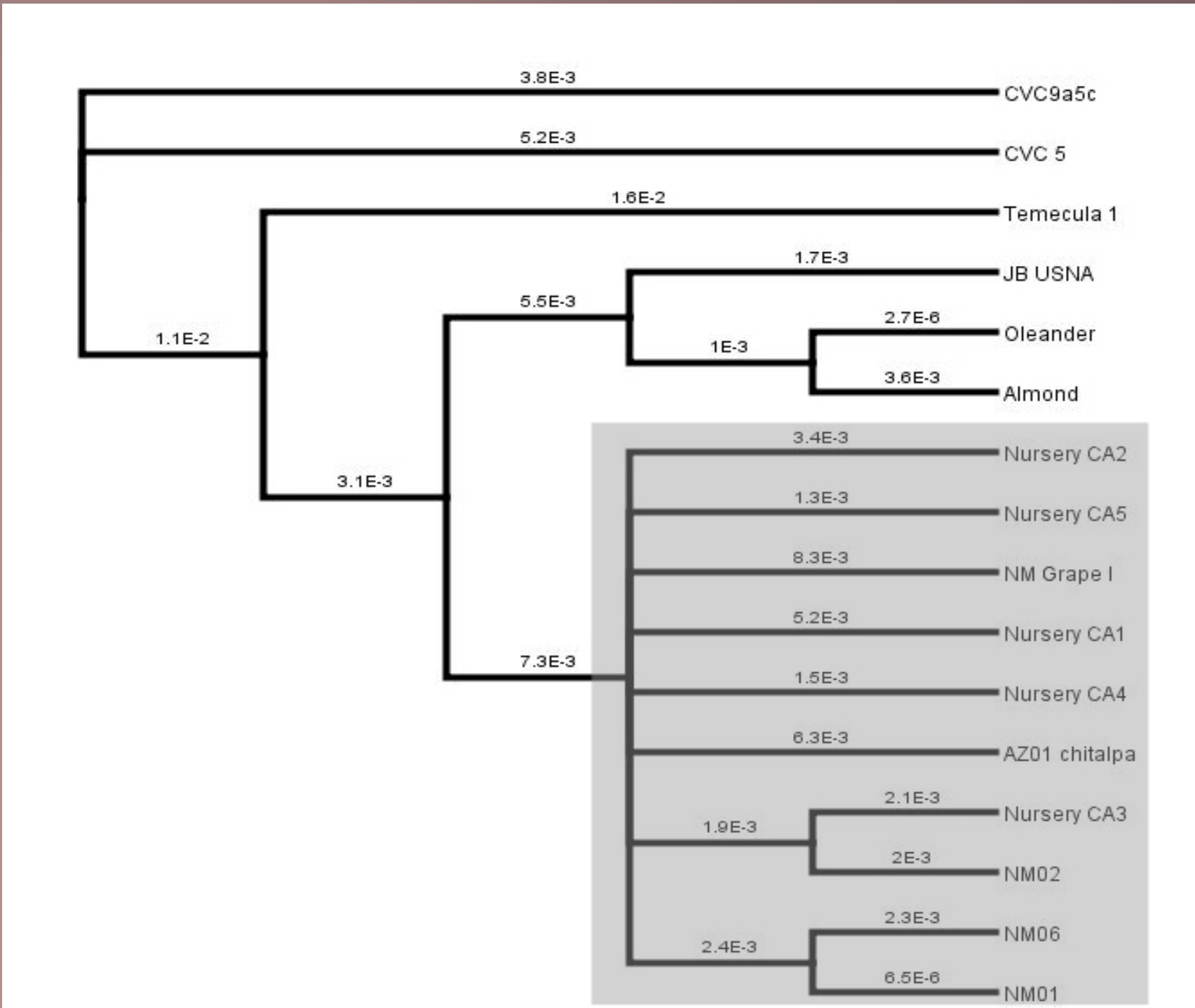
Chitalpa tashkentensis tree in Southern New Mexico exhibiting leaf scorch symptoms. (A and B) Leaf chlorosis and necrosis from symptomatic chitalpa trees. (C) Flowers from chitalpa (D) A symptomatic chitalpa tree exhibiting branch dieback.

	Chitalpa Sample	Sequence ID	ELISA	PCR	Bacterial Colony
	New Mexico				
1	FG1	-----	+	+	+
2	FG4	-----	+	No test	Not plated
3	MA1	NM01	+	+	+
4	WO1	-----	+	+	+
5	WO1a	-----	+	+	-
6	WO1b	-----	+	No test	Not plated
7	WO2a	-----	+	+	-
8	WO2b	-----	+	No test	Not plated
9	PB1	NM02	+	+	+
10	CBH	NM03	+	+	+
11	CB624	NM04	+	+	-
12	CB418	-----	-	-	-
13	CH33	-----	+	+	-
14	MA2	NM05	+	+	+
15	MA3	-----	+	+	+
16	VP	-----	+	No test	Not plated
17	MA6	NM06	+	+	-
18	HD3	NM07	+	+	-
19	BM1	-----	-	-	-
20	BM2	-----	-	-	-
21	SW1	NM08	+	+	+
22	SW5	NM09	+	+	-
23	MO1	NM10	+	+	-
24	FG2	NM11	+	+	-
25	FG3	NM12	+	+	-
26	WO2	NM13	+	+	+
27	LVC1	-----	+	-	-
28	LVC2	-----	-	+	-
29	CV1	-----	+	No test	Not plated
30	CV2	-----	Borderline	-	Not plated
31	CV3	-----	-	-	Not plated
32	HD3	-----	+	+	-
	ARIZONA				
33	SV1	AZ01	+	+	-
34	SV2	AZ02	+	+	-
35	SV3	AZ03	+	+	-
36	SV4	AZ04	+	+	-
37	SV5	AZ05	+	+	-
38	SV6	AZ06	+	+	-
39	SV7	AZ07	+	+	-
40	SV8	-----	-	-	Not plated

Data from southwest chitalpa samples. PCR was determined to be positive or negative by the presence of a product at the correct size on an agarose gel. The bacterial colony column refers to those samples which yielded *X. fastidiosa* colonies when cultured.



Amplified products from a subset of Arizona and New Mexico samples. (A) *X. fastidiosa* nested PCR products amplified from chitalpa trees were separated on a 1% agarose ethidium bromide stained gel. The resulting 450 bp band is denoted with an arrow. M is the molecular weight, A1-A3 are Arizona samples AZ1-AZ3 (see table 1), N1-N3 are New Mexico samples NM01-NM03 (see table 1) and - is a negative control.



Phylogram constructed with Geneious 2.5.3 illustrating the relationship between sequences of *X. fastidiosa* amplified from symptomatic chitalpa from Southern New Mexico (NM01 group, NM02 group, and NM06), Arizona (AZ01), and imported chitalpa trees from CA (CA1-CA5) found in nurseries versus other reported *X. fastidiosa* strains. The *X. fastidiosa* sequences used for the alignment for the following were all obtained from Genbank (www.ncbi.nlm.nih.gov) JB-USNA (genbank accession AY196792), almond strain (genbank accession AAAL02000008.1), oleander strain (genbank accession AAAM03000127.1) Temecula (genbank accession AE009442.1), and CVC strain 5 (genbank accession AF344191) and CVC 9a5c (genbank accession AE003849.1). Tree was constructed as a neighbor joining tree, Bootstrapped 1000 times using CVC strain 9a5c as the outgroup.

General Conclusions

❖ Presence of *X. fastidiosa* determined by ELISA, PCR, and culturing of bacteria.

❖ *X. fastidiosa* infected chitalpa trees distributed across the southwest.

❖ Highest frequency of *X. fastidiosa* infected chitalpa found in southern New Mexico and Sierra Vista, Arizona.

❖ Infected chitalpa is being imported into nurseries.

❖ Chitalpa trees may be a resevoir for *X. fastidiosa*.

Future Directions

➤ Testing of *X. fastidiosa* isolates for their potential to cause disease in other plant species such as grape, pecan, oleander, and alfalfa.

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