



Pierce's Disease Detected in New Mexico Grapevines

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Abstract

In the fall of 2006, grapevines from two separate vineyards in Southern New Mexico exhibited leaf scorch and dieback symptoms consistent with Pierce's disease. ELISA, PCR, and culturing assays all detected the presence of *Xylella fastidiosa*, the causal agent of Pierce's disease, in symptomatic tissue from both vineyards confirming that Pierce's disease is present in New Mexico. Preliminary phylogenetic analysis indicates that these New Mexico grape strains are closely related to *X. fastidiosa* strains recently found in chitalpa, an ornamental landscape tree, planted throughout the southwestern United States. This finding of Pierce's disease in New Mexico has prompted a larger survey of New Mexico vineyards to determine the extent of the disease in the state. Additionally, work is ongoing to determine the genetic relationship of strains from grape and chitalpa compared with known strains of *X. fastidiosa*.

INTRODUCTION

Xylella fastidiosa is a gram negative bacterium that lives endophytically within the xylem of plants species and is responsible for severe disease problems in many important crop and ornamental species. *X. fastidiosa* is transmitted by xylem feeding insect vectors such as the glassy-winged sharpshooter, leafhoppers, and spittle bugs (Redak, et al., 2004). Colonization of xylem by *Xylella fastidiosa* is thought to cause disruption of xylem function and associated disease symptoms which can include leaf necrosis, chlorosis, inedible fruit and eventual death of the plant (Hill and Purcell, 1995). Arid land agriculture is a major portion of New Mexico's economy and NM intensively grows several *X. fastidiosa* sensitive crops. In particular, NM has a robust grape and wine industry and is among the top producers of Pecans in the nation. In addition, oleander is a widely utilized ornamental in NM. Given the potential for serious disease problems and geographic proximity to other heavily affected areas, NM growers have been concerned about the potential for *X. fastidiosa* to establish itself in NM. Limited testing in previous years has never detected any *X. fastidiosa* infected plants (N. Goldberg, pers. Comm.) It has not been clear if the lack of *X. fastidiosa* caused disease in NM has been due to environmental conditions, the lack of suitable vectors, or simply the absence of the pathogen. We recently reported on the discovery of *X. fastidiosa* strains related to those known to cause Pierce's disease in chitalpa, a common landscape ornamental plant in southern NM. *X. fastidiosa* infected chitalpa are widespread in southern NM (manuscript in preparation) suggesting a potential reservoir of *X. fastidiosa* that could affect crop production in NM. In this report we show that Pierce's disease was detected in NM for the first time this year and that the strains of *X. fastidiosa* found in affected grapevines appear to be closely related to a *X. fastidiosa* strain we recently reported in landscape chitalpa plants (Randall et al, 2007).

Methods and Materials

Collection of grape samples. Samples from two different vineyards in Southern New Mexico were randomly selected in late September of 2006. The grape plants in these two vineyard exhibited leaf scorch type symptoms. Samples from these plants consisted of stems and leaves. The samples were placed in individual plastic bags which were labeled and stored at 4°C.

ELISA of symptomatic grape plants. The presence of *X. fastidiosa* was first determined by enzyme-linked immunosorbent assay (ELISA). To perform this assay, 0.3 grams of leaf petioles and the mid-veins were crushed using the mini-bead beater 96+ (Biospec products inc.) with 3 ml of extraction buffer (Agdia) at room temperature. ELISA assays were performed on 100 ul of extract according to kit instructions. ELISA plates were read on a Bio-Tek KC4 plate reader at 620 nm. At least four negative control samples were included in the plate. Test sample values were considered positive when they exceeded the negative control average by at least three times the standard deviation of the negative control samples, and borderline if they were at three standard deviations above the negative control average and negative when they were below three standard deviations of the negative control samples.

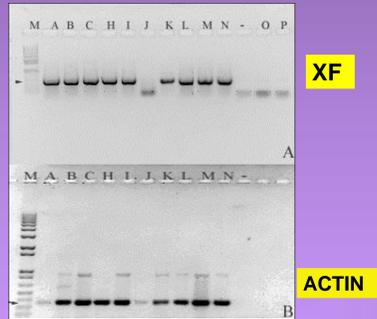
Bacterial plating from grape leaf tissue. The surfaces of the leaves were surface sterilized by submerging in 70% ethanol for two minutes followed by submerging the leaf in 30% bleach (1.5% sodium hypochlorite) for two minutes. The leaves were then rinsed in water twice. Leaf sections were then finely chopped on sterile filter paper and placed in an eppendorf tube with 600 microliters of sterile succinate-citrate-phosphate buffer. This is then ground by using a homogenizer for 30 seconds (Wistrom and Purcell, 2005 and Zintzun, 2006). Ten microliters of this extract is then added to 90 microliters of sterile succinate-citrate-phosphate buffer and plated on XfD2 media (Almeida et al., 2004). The plates were then placed in a 28°C incubator for three weeks prior to seeing growth.

Genomic DNA extractions from grape plants. Genomic DNA was extracted from grape plants using the Qiagen Plant DNeasy kit. The quality of the genomic DNA was verified on a 1% agarose gel and by amplification of actin as an internal control. Actin amplification was performed using actin gene specific primers, actin A:GGACTCTGGAGATGGTG; actin B:GCAGCTTCATCCGATC. 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, and two units of Taq Polymerase and 1ul of a 1:10 dilution of the genomic 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, 55°C for 45 seconds, and 72°C for 2 minutes, with a final elongation step of 72°C for 5 minutes. The 350 bp 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.

PCR analysis to determine the presence of *X. fastidiosa* with both genomic DNA preparations and Bacterial colonies. 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., 1997. The PCR components for this reaction consisted of 1X PCR Buffer (100mM Tris-HCl, 500mM KCl, pH 8.3), 1.5mM MgCl₂, 0.2mM dNTP's, 0.1 nmol of each primer, and two units (?) of Taq Polymerase and 1ul of a 1:10 dilution of the genomic DNA 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. The resulting products from the nested reaction and were then labeled using Big Dye Terminator (ABI) and purified using Bio Edge System columns prior to sequencing on ABI-3100 (NMSU-LiCor facility). The sequences were analyzed using the sequence scanner software (BioRad). Sequences of five of the nested PCR products obtained from the grape samples were analyzed using Blast from the NCBI website and Geneious Pro 2.5.3 for alignments and construction of phylogenetic trees.



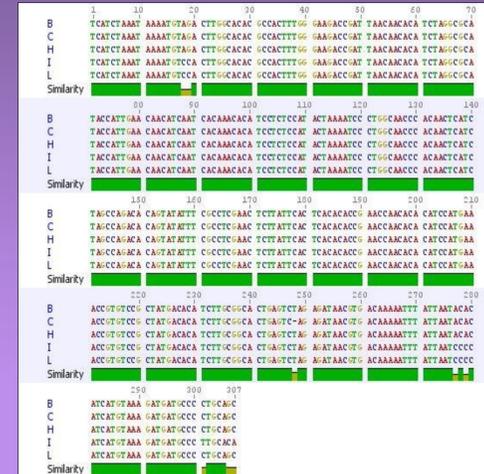
Symptomatic Chardonnay grape vine (A) and leaves (B) from a southern New Mexico vineyard. This vine was dead in September 2006.



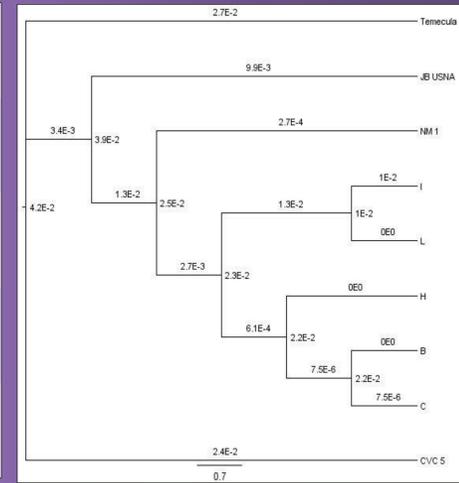
Amplified products from genomic grape samples. (A) *X. fastidiosa* nested PCR products amplified from grape samples were separated on a 1% agarose ethidium bromide stained gel. The resulting 450 bp band is denoted with an arrow. (B) Actin PCR products were separated on a 1% agarose ethidium bromide stained gel. The resulting 350 bp band is visualized with an arrow. M is the molecular weight, A through P are grape samples and - is a negative control.

SAMPLE	ELISA	PCR	Bacterial Colony
A	Borderline	Positive	Positive
B	Positive	Positive	Positive
C	Negative	Positive	Positive
H	Positive	Positive	Positive
I	Negative	Positive	Negative
J	Positive	Negative	Negative
K	Borderline	Positive	Negative
L	Positive	Positive	Negative
M	Positive	Positive	Negative
N	Positive	Positive	Negative
O	Not tested	Negative	Negative
P	Not tested	Negative	Negative

Data from symptomatic grape samples. The ELISA test was considered positive if the absorbance (620 nm) as read by the plate reader was above 3 standard deviations from the average of the four negative controls on the ELISA plate. A borderline result indicates that the absorbance was right at the three standard deviation cutoff. Negative results indicate that the absorbance was below the three standard deviation cutoff mark. 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.



Multiple sequence alignment indicates similarity and differences in DNA sequences of nested PCR products amplified from symptomatic grape samples. Alignment performed using Geneious 3.3.5.



Phylogram illustrating relationships among sequences of *X. fastidiosa* amplified from symptomatic grape samples in southern New Mexico (B, C, H, I, and L) versus other reported *X. fastidiosa* isolates. Sequences from NM grape samples are most closely related to NM 1 isolate from chitalpa.

General Conclusions

❖ First description of *Xylella fastidiosa* in New Mexico.

Presence of XF in NM grape demonstrated by ELISA, PCR, and growth of XF bacterial colonies.

❖ Blast Analysis of PCR products indicate that the XF found in infected NM grape are most closely related to the NM-1 isolate from Chitalpa.

Future Directions

- ❖ Intensive monitoring of NM vineyards.
- ❖ Insect surveys to determine if there is an insect vector population in southern NM.
- ❖ Screening potential reservoirs of XF such as Chitalpa.
- ❖ Testing of NM XF isolates for their potential to cause disease in other species such as pecan, oleander, and alfalfa.

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