First Report of Pierce’s Disease in New Mexico

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Abstract
In the fall of 2006, Vitis vinifera Chardonnay grapevines from two 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 Pierce’s disease in New Mexico. Phylogenetic analysis indicates that isolates from New Mexico grapes are closely related to a X. fastidiosa isolate recently found in the landscape plant Chitalpa tashkentensis in New Mexico.

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, other sharpshooters, and spittle bugs (7). Colonization of xylem by X. 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 (2). While X. fastidiosa is a benign endophyte in some plant species this new world pathogen causes severe disease in a number of economically important crop species (5). In the United States and Central America, X. fastidiosa causes diseases on grape, coffee, pecan, and citrus. Between 1884 and 1896 X. fastidiosa, the causative agent of Pierce’s Disease, destroyed nearly 35,000 acres of grapes and at least 50 wineries closed in California due to this disease (9). One hundred and twenty years later Pierce’s disease is still one of the primary concerns for US wine producers in California and Texas.

Semi-arid land agriculture is a major portion of New Mexico’s economy and New Mexico produces several X. fastidiosa sensitive crops. In particular, New Mexico has a robust grape and wine industry, is among the top producers of pecans in the nation, and has extensive oleander plantings in ornamental landscapes. Given the potential for serious disease problems and geographic proximity to other heavily affected areas, there is concern about potential introductions of X. fastidiosa into New Mexico. Limited testing in previous years had never detected any X. fastidiosa infected plants (N. Goldberg, personal communication). It has not been clear if the lack of X. fastidiosa-caused disease in New Mexico has been due to environmental conditions, the lack of known suitable vectors, or simply the absence of the pathogen. We recently reported on the discovery of X. fastidiosa in chitalpa (Chitalpa tashkentensis), a common landscape ornamental plant in southern New Mexico (6). X. fastidiosa infected chitalpa are widespread in southern New Mexico, suggesting a potential reservoir of X. fastidiosa that could affect nearby crops and ornamentals. In this report we show that Pierce’s disease of grape was detected in New Mexico for the first time in 2006 and that the isolates of X. fastidiosa found in affected grapevines appear to be closely related to a X. fastidiosa isolate we recently reported in landscape chitalpa plants in New Mexico (6).
Identification of *X. fastidiosa* in New Mexico Grape

Chardonnay grapes from two different vineyards (several miles apart) in southern New Mexico exhibiting leaf scorch and vine dieback symptoms were sampled in the late summer and early fall of 2006. One symptomatic chardonnay vine died within a month of our first sampling (Fig. 1). Samples were collected from symptomatic plants from the two vineyards in late September 2006 and analyzed for *X. fastidiosa*. The grape samples were first analyzed by enzyme-linked immunosorbent assay (ELISA) (Agdia Inc., Elkhart, IN). ELISA analysis of the grape samples were positive in samples B, H, J, L, M, and N, borderline positive in samples A and K, and negative for samples C, and I.

Fig. 1. Symptomatic Chardonnay grape vine (A) and leaves (B) from a southern New Mexico vineyard on September 2006. This vine was dead in September 2006.

PCR analysis used total DNA isolated from grape using the Plant DNAeasy kit (Qiagen Inc., Valencia, CA). The suitability of total DNA for PCR amplification was verified by amplification of the grape actin gene using actin specific primers. The expected 350 bp band for actin was detected in all grape samples indicating that the DNA preparations were of sufficient quality for amplification (Fig. 2a, data not shown for samples O and P). The actin primers only amplify actin from the plant DNA since there is very limited homology between eukaryotic actin and bacterial actin homologues (8).
Subsequent PCR analysis was performed using previously described nested PCR primers designed to detect all known strains of *X. fastidiosa* (4). A 450 bp product indicative of *X. fastidiosa* was detected in samples A, B, C, H, I, K, L, M, and N (Fig. 2b). These products were directly sequenced and BLAST analysis showed similarity to other *X. fastidiosa* sequences in GenBank. Two samples (C and I) were negative for *X. fastidiosa* by ELISA but were positive by PCR amplification and a number of other samples were borderline for *X. fastidiosa* by ELISA but positive by PCR analysis. This is likely due to the difference in sensitivity between the ELISA and PCR assays for detecting *X. fastidiosa*. Previously, the ELISA assay was estimated to have a limit of detection of approximately $10^4$ bacteria per ml while the nested PCR assay used in this work was reported to detect as few as five bacterial cells in a sample (4). Therefore, these contradictory results may reflect the different sensitivities of the ELISA and PCR assays. One grape sample (J) was positive for *X. fastidiosa* by ELISA analysis but was negative by PCR analysis. We have found that the presence of the pathogen, *X. fastidiosa*, appears to vary spatially within the same plant host (data not shown) and it is therefore possible that the inconsistent results for this sample reflect this variability.

The presence of *X. fastidiosa* was also assayed by culturing (10). Ten microliters of sample extract was added to 90 microliters of succinate-citrate-phosphate buffer and plated on XFD2 media (1). Plates were incubated at 28°C and monitored for colony development over the course of 4 to 6 weeks. Bacterial colonies were obtained from samples A, B, C, and H. These colonies were first visible as small white colonies 20 days after plating on XFD2 media. Colonies were deemed to be *X. fastidiosa* based on the results of whole cell PCR assays using the nested PCR primers (data not shown).

**Sequence Analysis**

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 (BioMatters, Auckland, New Zealand) for alignments and construction of phylogenetic trees. This analysis indicates that the sequences have limited diversity from each other (Figs. 3 and 4). Samples B, C, and H have essentially the same sequence except with one base difference. Samples I and L have essentially the same sequence save for three base pair differences when compared to one another. Samples I and L are different than the sequences obtained for samples B, C, and H by at least five nucleotides (Fig. 3).
Fig. 3. Multiple sequence alignment indicates similarity and differences in DNA sequences of nested PCR products amplified from symptomatic grape samples.

Fig. 4. 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 New Mexico grape samples are most closely related to New Mexico 1 isolate from chitalpa.
Blast analysis (www.ncbi.nlm.nih.gov) indicated that the grape sequences from all of the samples are 97% similar to X. fastidiosa from Japanese Bonsai (JB-USNA genbank accession AY196792), 96% similar to the wine grape isolate Temecula1 (genbank accession AE009442.1) which is known to cause Pierce’s disease in grapes, and 95% similar to a citrus isolate CVC 5 (accession AF344191) that causes citrus variegated chlorosis (CVC).

Alignment of the New Mexico grape X. fastidiosa sequences with six other X. fastidiosa isolates was performed to examine phylogenetic relationships. The X. fastidiosa sequences used for the alignment for the following were all obtained from Genbank (www.ncbi.nlm.nih.gov): JB-USNA, Temecula 1, CVC 5, and NM 1 (NM chitalpa isolate, accession EF109936) (6). Tree was constructed as a neighbor joining tree using CVC 5 as the outgroup. While this is a limited analysis based on a single sequence, all X. fastidiosa sequences from New Mexico grape samples were most closely related to the NM Chitalpa isolate (Fig. 4). While these data indicate that the New Mexico isolates are clearly most closely related to the NM chitalpa isolates it is unclear from this limited analysis whether the New Mexico grape X. fastidiosa isolates are considered to be "grape strain-specific" X. fastidiosa such as Temecula. There are other examples of non-grape strains, such as the coffee and CVC strains, which induce Pierce's disease in grapes (3). Additional detailed molecular studies are underway to further characterize the X. fastidiosa isolates present in New Mexico.

There are several scenarios with which X. fastidiosa may have been introduced into southern New Mexico. First, it is possible that the grape stock which was imported and planted in the vineyards was infected prior to planting. This is considered unlikely since the grapevines were planted anywhere from 5 to 25 years ago (B. Maier, personal communication). Had the vines been infected upon planting it is doubtful that they would have survived to the present. A second scenario involves the landscape chitalpa plants which are widely distributed in the Southwest. The chitalpa plant is not native to southern New Mexico and thus has been imported from other states. We have recently detected X. fastidiosa in imported chitalpa nursery stock indicating it is being shipped to New Mexico already infected with X. fastidiosa (unpublished data). It is possible that these infected chitalpa are a reservoir which either persists over time in established landscape plants, is renewed by perpetual importation of infected stock, or both. The similarity between isolates and the occurrence of X. fastidiosa in vineyards established 20 or more years ago argues that these chitalpa are the reservoir for this newly detected Pierce’s disease outbreak. Another scenario is that an insect vector population has introduced the pathogen into southern New Mexico from any of the states bordering New Mexico, although the unique nature of the New Mexico X. fastidiosa isolates argues against this. While it appears that X. fastidiosa is being transmitted among plants in New Mexico no stable population of transmission competent insects is known to exist in southern New Mexico. Further studies of potential vectors and their dynamics will be necessary to further resolve how X. fastidiosa was introduced into New Mexico and how it may spread throughout the state in the future.

This report describes the first known outbreak of Pierce’s disease in New Mexico. Multiple independent lines of testing all identified the presence of X. fastidiosa in symptomatic vines in two vineyards located in southern New Mexico. The long term effects of this outbreak are unknown. Current and future work includes more intensive monitoring of vineyards to establish the scope of the outbreak and detect any local spread if it occurs, further work on examining potential reservoirs of X. fastidiosa including chitalpa, and insect surveys to determine if there is a vector in southern New Mexico which could cause further spread of Pierce’s disease in New Mexico. Additional work also includes detailed molecular phylogeny studies to better characterize New Mexico X. fastidiosa isolates, and the testing of X. fastidiosa isolates currently existing in New Mexico for their potential to cause disease in other species important to the region such as pecan, alfalfa, and oleander.
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Literature Cited