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 Gram-negative bacterium known to cause disease in many diverse plant species, was detected in chitalpa trees. At the same time, X. fastidiosa was detected for the first time in grapevine in New Mexico. The common use of chitalpas 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 chitalpas and to what extent the chitalpa isolates of X. fastidiosa will affect other trees.

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 systemic feeding insects such as spittlebugs, adelgids, and leafhoppers [2]. Historically X. fastidiosa included Pseudomonas in its genus [1], when xylem-fixing strains were switched to X. fastidiosa (X. fastidiosa 1975. 72°C for 2 minutes, and 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

Methods and Materials

Collection of chitalpa and dieback samples. Leaves from established chitalpas exhibiting leaf scorch type symptoms from throughout New Mexico during the summer and fall of 2006. Chitalpa samples were also collected from Tucson and Sierra Vista Arizona. Environmental samples in southern New Mexico in October of 2006. Samples from these plants consisted of leaves, branches, and flowers.

ELISA of symptomatic chitalpas plants. The presence of X. fastidiosa was first tested for by enzyme-linked immunosorbent assay (ELISA). Two different methods were utilized for this study. First, 64 grams of leaf and flowers and the mid-viruses were planted in plant samples weighing 5-6 g of extraction buffer 2 (Agdia, Inc. Elkhart IN) and the tissue was ground on sterile filter paper and placed in an eppendorf tube with 600 microliters of sterile succinate-citrate-phosphate buffer. Leaf pieces were ground in a microcentrifuge, and the resulting 350 bp actin band was then visualized on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light with the Kodak 2000R Station (Eastman Kodak

Chitalpa mid-vein tree in Southern New Mexico exhibiting leaf scorch symptoms. (A) Polyacrylamide gel and sequencing for comparative genomics. (B) Chitalpa trees (C) A symptomatic chitalpa tree exhibiting branch death.

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 on an agar plate filled with 2 ml of nutrient media (tryptophan-yeast-glucose medium 30:18.6:1.2, pH 7.0). Leaf sections were then submerged in 2 ml of nutrient media (tryptophan-yeast-glucose medium 30:18.6:1.2, pH 7.0). Leaf pieces were ground in a microcentrifuge, and the resulting 350 bp actin band was then visualized on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light with the Kodak 2000R Station (Eastman Kodak

Data from southwest chitalpa samples. PCR was determined to be positive or negative by the presence of a 350 bp band after visualization of the gel. The bacterial colony culture revealed those samples which yielded X. fastidiosa colonies when cultured.

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 reservoir 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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