Optimized diagnostics of Xylella fastidiosa in pecan with impacts on domestic and international distribution

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Xylella fastidiosa is the causal agent of PBLS

• What is PBLS?
  • A chronic bacterial disease that can cause major yield losses in susceptible pecan cultivars.

• Symptoms of PBLS
  • Begins with necroses of leaflet tips and margins, later progressing in a uniform pattern toward the leaf base and midribs.
  • Lesions are usually tan to light brown in color when spreading through leaflet tissue.
  • Defoliation can be severe and may occur on individual limbs or systemically across the entire plant.
The biology of *X. fastidiosa*

- *X. fastidiosa* is a xylem-limited fastidious, rod-shaped bacteria

- Can infect at least 309 different plant species, including grape, peach, citrus, almond, oleander, sycamore, coffee, and olive

- Five strains (subspecies) have been identified based on a distinctive, non-overlapping host-range
  - Pecan pathogen is a member of subspecies multiplex

Case Study: Olive Quick Decline Syndrome (OQDS)

- X. fastidiosa-infected coffee plants were introduced to Leece province, Italy between 2008-2010.

- Already spread through Europe, infecting over one million olive trees.

- Symptoms that include leaf scorching, twig and branch dieback and, ultimately, tree death.

- X. fastidiosa has been labeled a quarantine organism by EPPO (EU Directive 77/93).
What is the potential impact of PBLS in pecan?

• In severe conditions, Cape Fear trees were reported to have up to 58% defoliation at the end of the season when compared to non-infected trees.
  • 24% reduction in terminal weight
  • 10-13% reduction in nut weight
  • 14-19% reduction in kernel weight
  • 12% yield loss, a value that could lead to losses of over $466/ha.

• Unknown economic impact in other cultivars

Cape Fear cultivar showing symptoms of PBLS. Rebecca A. Melanson, Mississippi State University Extension, Bugwood.org
Modes of transmission

• The primary mode of transmission of *X. fastidiosa* is through xylem-feeding insects.
  • Spittlebugs
  • Sharpshooters

• PBLS can be transmitted via grafting

• PBLS found in progeny of infected maternal trees
Are current diagnostics methods reliable for PBLS detection?

• The USDA-ARS Pecan Breeding and Genetics Program found inconsistencies in *X. fastidiosa* detection results.

• Highlighted the need for optimized protocols.

• Types of tests
  • Serological methods → ELISA
  • Molecular methods → PCR/qPCR, sequencing
USDA-ARS Pecan Breeding and Genetics Program

• National Collection of Genetic Resources for Pecans and Hickories

• Mission:
  • develop superior pecan cultivars and rootstocks
  • determine heritability constants for superior tree and nut characteristics;
  • develop host plant resistance to control pecan insects and diseases;
  • effectively collect, document, preserve, evaluate, enhance, and distribute pecan and hickory genetic resources
Goals of this study

- To validate and optimize diagnostic protocols of ELISA and PCR for detection of X. fastidiosa in pecan plant tissues

- To screen pecan cultivars and varieties in Texas for the presence of PBLS

- To identify other species of Carya (hickories) that may be susceptible to X. fastidiosa infection.
In Texas and Indiana, we collected 13 species of *Carya*

| Species                  | Common Name                   | Location                                                              | Quantity |
|--------------------------|-------------------------------|                                                                      |----------|
| *Carya illinoinsensis*   | Pecan                         | Somerville, Brownwood, Medina and Uvalde Counties, TX                 | 130      |
| *C. pallida*             | Sand Hickory                  | Daviess County, IN                                                   | 1        |
| *C. pallida x C. tomentosa* | Sand Hickory x Mockernut     |                                                                      | 1        |
| *C. tomentosa*           | Mockernut                     |                                                                      | 1        |
| *C. cordiformis*         | Bitternut                     | Somerville, TX; Daviess County, IN                                   | 3        |
| *C. cathayensis*         | Chinese Hickory               |                                                                      | 5        |
| *C. floridana*           | Scrub Hickory                 |                                                                      | 2        |
| *C. laciniosa*           | Shellbark Hickory             |                                                                      | 1        |
| *C. glabra*              | Pignut Hickory                |                                                                      | 2        |
| *C. aquatica*            | Water Hickory                 | Somerville, TX                                                       | 2        |
| *C. ovata*               | Shagbark Hickory              |                                                                      | 1        |
| *C. palmeri*             | Mexican Hickory               |                                                                      | 1        |
| *Platycarya strobilacea* | Platycarya                    |                                                                      | 1        |
| *Pterocarya stenoptera*  | Chinese wingnut               |                                                                      | 1        |
1. **Add samples to 96-well plate pre-coated with capture antibody**

   ![Capture antibody](image)

2. The Xylella-specific target protein (antigen) binds to the antibody

   ![Target Xylella protein (antigen)](image)

3. A second antibody bearing an enzyme conjugate is added to the plate, which will bind to the antibody-antigen complex

   ![Antibody enzyme conjugate](image)

4. A peroxidase substrate is then added and produces a signal

   ![Peroxidase substrate](image)

5. Signal intensity is measured by a plate reader at 650 nm
Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA)

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Optimization of ELISA diagnostics

• Can we improve reliability of PBLS diagnostics?
• How do sample preparation procedures impact results?
Comparison of sample preparation procedures

- Three pecan cultivars were sampled and subject to different sample preparation procedures.

- Percent positive:
  - Extracted sap – 100%
  - Incubated petioles – 69.2%
  - Homogenized tissue – 30.7%
Detection of PBLS by ELISA in Texas pecans

• Revised diagnostics methods of ELISA have identified 14 trees to be positive for X. fastidiosa in Texas.

![Detection of X. fastidiosa in 152 Carya samples by ELISA](graph.png)
Collect samples exhibiting symptoms of PBLS. Store at 4°C with adequate humidity.

Isolate DNA using extraction buffer or previously published protocols.

Prepare PCR reaction with extracted DNA as template. Perform program in thermal cycler.

Run PCR product (amplicon) through gel electrophoresis.

Check agarose gel under UV light for X. fastidiosa specific band.
PCR diagnostics

- PCR detection was performed using sap, extracted DNA, and/or endosperm.

- Three different molecular markers (primer sets) were used to detect for X. fastidiosa.
  - (B) RST (70-sigma factor)
  - (C) 16s rRNA
  - (D) HL (hypothetical protein)
PCR can be verified by sequencing

- Twenty geographically distinct Carya samples were selected for sequencing of 16S rRNA PCR fragments
- Sequences were compared to known X. fastidiosa DNA in NCBI Genbank
PBLS is present in southern and western pecan growing regions

• Southern Region
  • PBLS was first reported in Louisiana by Sanderlin and Heyderich-Alger (2000).
  • We detected in X. fastidiosa 130 pecan (C. illinoinensis) samples in Texas (100% positive).

• Western Region
  • New Mexico → 95/162 pecans were positive by ELISA (58.6% positive)
    • Jason French, Plant Diagnostic Clinician, New Mexico State University
  • California → 13/20 pecans were positive by ELISA (65% positive)
    • Jason French, Plant Diagnostic Clinician, New Mexico State University
  • Arizona → 92/130 pecans were positive by ELISA (71% positive, 39% asymptomatic pecans were positive for Xf)
    • Josh Sherman, Extension Agent Assistant, University of Arizona
Management strategies

• Insect vector management

• Reduce weeds and wild grasses in orchards

• Avoid introducing contaminated plant material

• There are little to no control methods for eliminating the X. fastidiosa from pecan.

• No resistant cultivars have been discovered.
Hot-water treatments can be used to sterilize contaminated graft wood

• Do not knowingly distribute contaminated plant material without prior sterilization.

• Steps:
  1. Soak graft wood in 115°F water for 30 min
  2. Transfer to room temperature water for 1-2 min
  3. Make sure to completely submerge the graft wood during treatment!

• Found to be 97% effective in preventing graft-transmission of X. fastidiosa

Resources

• Understanding and Managing Pecan Bacterial Leaf Scorch (sepga.com)
  • Rebecca A. Melanson, Extension Plant Pathologist, Central MS Research and Extension Center

• Pesticides Database (pecan.ipmpipe.org)
  • Bill Ree, Extension Program Specialism II, Texas A&M Agrilife Extension

• National Plant Diagnostic Network
  • SPDN (spdn.org)

• Cooperative Extension System (CES) (nifa.usda.gov)

Southern Plant Diagnostic Network (SPDN)
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