

TITLE: Incidence and Distribution of Bacterial Leaf Scorch of Hardwoods in the North Central and Plains States **Progress for 2008**

LOCATION: MI, WI, MN, IA, IN, IL, MO, KS, NE, SD, ND

DURATION: 3-year project **FUNDING SOURCE:** Base EM

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PROJECT OBJECTIVES: 1. Determine the incidence of Bacterial leaf scorch (BLS) in species of *Quercus*, *Acer*, *Platanus*, *Ulmus*, *Morus*, *Tilia* and other hardwoods. 2. Determine the distribution of BLS in the 11 North Central and Plains States. 3. Relate the occurrence of BLS to mapped landscape-scale physiographic, edaphic, and climatic data.

JUSTIFICATION: This project directly addresses many of the emphases of the 2008 Evaluation Monitoring selection criteria as below:

- *Geographic scale:* This project has a broad geographical range as susceptible tree hosts are wide-spread throughout the North Central and Plains States. Additionally, the project will examine natural, rural and urban forests. The pathogen has a broad hardwood host range. Multiple cooperators in the States will be involved with this project.
- *Biological impact/political importance:* Susceptible oak species are a particularly significant component of natural, rural, and urban forests throughout the region, as are *Acer* and *Ulmus* species. BLS is a significant, as yet unknown mortality agent, directly and indirectly causing *Tree Mortality*. More often it decreases vigor and increases susceptibility to drought damage. Thus, it can be a component of widespread oak decline and decline in other species and may result in reduction of timber resources.
- *Drought:* The leaf scorch, early autumn dormancy, and decline symptoms associated with BLS mimic those of drought stress. BLS symptoms increase with moisture stress. We will use detection of the causal agent, *Xylella fastidiosa*, as a method of differentiating the effects of drought stress alone from disease, and will compare the results to the landscape-scale data.
- *Climate change:* Distribution of BLS mapped to the landscape-scale data will provide the basis of predicting expansion of the geographical range for the disease with climate change. Distribution of BLS is considered to be cold-limited and the target tree species are widespread in the mid-west. The consequences of climate change are predicted to affect the mid-west climate with warming and drying.
- *Poor crown condition:* Detection of infection by *X. fastidiosa* may be a critical variable in determining the incidence of oak decline. Uncompacted live crown ratio and percentage crown dieback in trees showing leaf scorch with and without *X. fastidiosa* infection will be compared to clarify the relationship of BLS to crown condition and decline.

- *Insects and diseases*: Documenting the incidence and distribution of BLS will improve understanding of hardwood decline and susceptibility to insects and other diseases. Infection with *X. fastidiosa* is documented as increasing susceptibility of *Ulmus* spp. to Dutch elm disease, increasing dieback in *Platanus* with anthracnose, and causing decline in vigor, and sometimes mortality, in many hardwoods.
- *Validating insect and disease risk models*: Insect and disease risk models could be impacted by the incidence and distribution of BLS. This study should supplement and improve some risk models.

DESCRIPTION:

a. Background: Bacterial leaf scorch of shade trees is the common name for a disease caused by *Xylella fastidiosa*, a xylem-inhabiting bacterium that has fastidious nutritional requirements and is difficult to culture or verify by culturing. The disease affects forest trees (including oak, sycamore, elm, and maple) throughout the eastern and southeastern U.S. as well as in parts of the Midwest. In New Jersey, BLS was first detected in populations of trees in the red oak group in several western counties 20 years ago and since has spread throughout the state, affecting as many as 44% of susceptible oaks in some communities. Diagnosis and detection recently depended on ELISA immunology assays but have just been improved 100-fold in sensitivity with the development of Real-Time PCR methodology (rt-PCR). Population increases of *X. fastidiosa* in vessel elements lead to water stress symptoms, especially chlorosis followed by necrosis of leaf margins and interveinal areas, leaf curling, decreased seed production, delayed budbreak, early autumn dormancy, decline, dieback, and sometimes mortality. Increasing incidence and distribution of BLS combined with drought will increase decline and mortality in susceptible hardwoods. Introduction of exotic leafhopper vectors can increase incidence and distribution of BLS.

b. Methods: Cooperators and volunteers will locate trees showing leaf scorch during August and September. Five living leaves with and without symptoms will be collected from a tree and placed in a self-addressed stamped Tyvek® envelop for shipment. Approximate tree location will be recorded by GPS coordinates or street address. Diameter at breast height will be measured. Condition of the tree crown will be measured as uncompact live crown ratio and percentage of crown dieback. Information will be recorded on or in the envelopes. Leaf samples from a minimum of 100 trees per state (1100 to 2000 trees) will be photographed then processed to extract DNA from xylem tissues using magnetic bead capture microcolumns. Quality of DNA and presence of *X. fastidiosa* will be determined with rt-PCR technology using *X. fastidiosa* specific primers. Three replicates of each sample will be assayed. Positive and negative trees will be mapped for distribution. Climatic (especially isotherm lines), physiographic, and edaphic data will be overlaid on the final maps and correlations examined.

c. Products: This project will produce 1) quantitative assessment of the relationship between infection by *X. fastidiosa*, leaf scorch, and crown condition; 2) maps of states showing incidence and distribution of BLS in select hardwood species; 3) predictive understanding of the relationships between BLS distribution and climatic, physiographic, and edaphic parameters; and 4) a peer-reviewed publication.

d. Schedule of Activities: Year 2- Re-organize cooperators in spring and summer. Re-organize collection to optimize distribution and coverage. Process samples using both ELISA and real-time PCR analyses. Map distribution and incidence data and overlay maps with landscape-scale data. Year 3- complete synthesis and analysis of data and write a final manuscript.

e. Progress/Accomplishments:

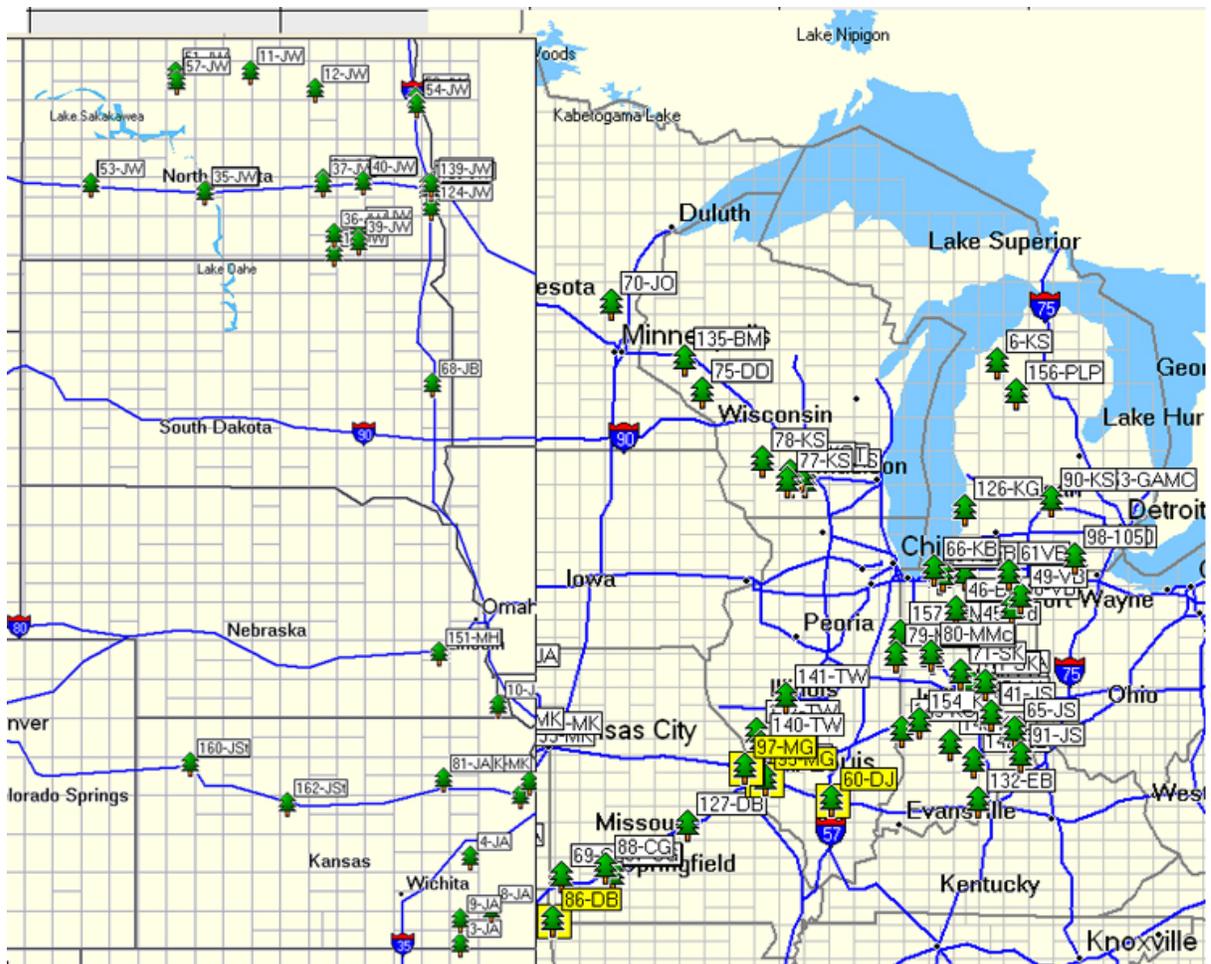
In 2008 we processed over 160 samples of leaves and twigs from *Quercus* spp. and other species showing scorch symptoms on the leaves. The tree species and the numbers of samples collected from each species included the following: *Acer ginnala* (Amur maple) 2, *Acer negundo* (Box elder) 2, *Acer platanoides* (Norway maple) 3, *Acer rubrum* (Red maple) 1, *Acer saccharinum* (Silver maple) 1, *Acer saccharum* (Sugar maple) 1, *Acer tataricum* (Tatarian maple) 1, *Aesculus glabra* (Ohio Buckeye) 3, *Aesculus hippocastanum* (Buckeye Horsechestnut) 4, *Cercis occidentalis* (Western redbud) 1, *Fraxinus pennsylvanica* (Green ash) 9, *Juglans nigra* (Black walnut) 1, *Malus* spp. (Flowering crabapple) 1, *Morus rubra* (Mulberry) 3, *Parthenocissus quinquefolia* (Virginia creeper) 1, *Populus tremula* 'Erecta' (Columnar poplar) 2, *Populus tremuloides* (Quaking aspen) 2, *Prunus virginiana* (Chokecherry) 1, *Pyrus ussuriensis* (Prairie gem) 3, *Quercus alba* (White oak) 1, *Quercus bicolor* (Swamp oak) 1, *Quercus imbricaria* (Shingle oak) 1, *Quercus macrocarpa* (Bur oak) 12, *Quercus palustris* (Pin oak) 8, hybrid *Quercus rober* 'Fastigiata' x *Q. bicolor* ('Regal Prince') 1, ***Quercus rubra* (Red oak) 77**, *Quercus velutina* (Black oak) 3, *Syringa villosa* (Late or Villous lilac) 1, *Syringa vulgaris* (Common lilac) 1, *Tilia americana* (American linden) 8, *Ulmus americana* (American elm) 5, *Ulmus davidiana* var. *japonica* (Japanese elm) 1, *Vitis* sp. 1.

Each sample was tested twice using two different assay methods. Real-time PCR was used to assay each sample and the reaction run included positive *X. fastidiosa* control, negative *X. fastidiosa* oak-leaf DNA control, and a supplemental pair of primers for an oak gene for quantification. A positive serological control, supplied by the manufacturer AgDia, and *X. fastidiosa* positive oak-leaf control were used with ELISA assays.

Positive samples were from the Noel, Missouri to St. Louis and nearby suburbs as well as from central Illinois, Mt. Vernon, IL.

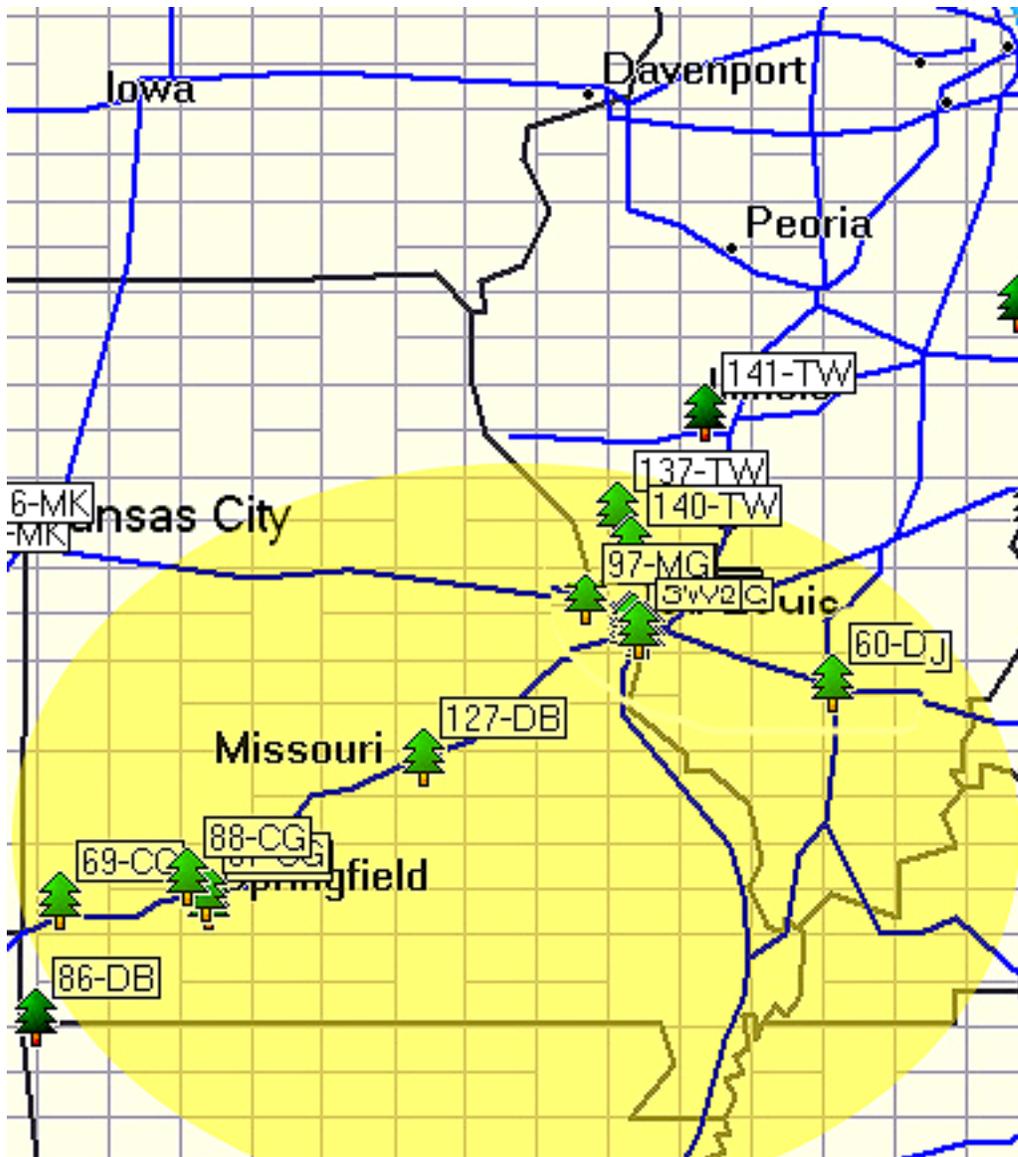
Samples were received from 10 of the 11 states and their locations according to supplied GPS coordinates or street addresses are mapped on the Garmin MapSource software from which the below images are taken. Yellow flags are positive samples for *X. fastidiosa*. The yellow circle outlines a zone expected to have high incidence of Bacterial Leaf Scorch and this zone may extend into Kansas and possibly southwestern Ohio. Positive samples from Oklahoma and Kentucky have been reported previously.

Iowa did not participate this year. So next year, we will put extra effort in obtaining samples from Iowa, Minnesota, Nebraska, and South Dakota.



Distribution of samples of leaf scorch received in 2008. There may be several samples beneath the flag-label of each visible flag. **Yellow flags** are samples positive for *X. fastidiosa* and Bacterial Leaf Scorch disease.

Expected distribution of *Xylella fastidiosa* in *Quercus* spp. in North Central and Plains states.



COSTS: Year 2

	Item	Requested FHM EM Funding	Other-Source Funding	Source
YEAR 2				
Administration	Salary	\$18,550	\$25,810	0.4 FTE MSU %FTE
	Fringe	\$6,360	\$ 9,713	0.4 FTE MSU
	Overhead	\$0		
	Travel	\$2,090		
Procurements	Contracting			
	Equipment			

	Supplies	\$8,000		
	Total	\$35,000	\$35,523	

	Item	Requested FHM EM Funding	Other- Source Funding	Source
YEAR 3	All			
	Total	\$0		

Budget Narrative: *Salary and fringes* are for a Research Associate for 4-months to do DNA extractions, real-time PCR and ELISA analyses of all the samples from the 11 States. *Travel* will include within Michigan for collection of samples and also into other States whenever cooperators are unable to cover an important portion of their state. *Supplies* include self-addressed stamped envelopes (500 -1000) and the costs of other printing and postage. Additionally, the expensive reagent kits for Sybr-Green fluorescent dyes for real-time PCR analyses, PCR-columns for cleaning DNA preparations [ca. \$1/sample for 1000 samples], ELISA diagnostic kits from AgDia Corp., agarose and PCR reagents and miscellaneous plastic ware and chemicals.

The Michigan Department of Natural Resources Forest Health Program will assist in field sample and data collection.

USFS S&PF, St. Paul will assist with field sample and data collection and analysis.