CHAPTER 10.

Bacterial Leaf Scorch Distribution and Isothermal Lines

(PROJECT NC-EM-08-02)

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INTRODUCTION

Bacterial leaf scorch (BLS) 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. Forest trees including oak, sycamore, elm, planetree, sweetgum, mulberry and maple are species susceptible to *Xylella* infection (McElrone and others 1999) throughout the Eastern and Southeastern United States. It is not yet known how common and widespread BLS is in trees in the North Central States (Iowa, Illinois, Indiana, Michigan, Minnesota, Missouri, and Wisconsin) and Plains States (Kansas, Nebraska, North Dakota, and South Dakota). 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 percent of susceptible oaks in some communities (New Jersey Forest Service 2002). Population increases of *X. fastidiosa*, production of unidentified toxins (Hayward and Mariano 1997), xanthan-like gums, and biofilms in vessel elements lead to water stress symptoms (Simpson and others 2000), 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 (Barnard and others 1988, Lashomb and others 2002). Increasing incidence and distribution of BLS combined with drought will increase decline and mortality in susceptible hardwoods. Moisture stress increases the expression of symptoms of BLS. *Xylella* is vectored by various insects in the Homoptera family including sharpshooter leafhoppers and spittlebugs (Pooler and others 1997). Introduction of new vectors that are more efficient in transmitting the pathogen can increase the economic damage caused by the disease as occurred in California when the glassy-winged sharpshooter increased the incidence of the *X. fastidiosa*-induced disease, Pierce's disease, which has been threatening the grape crop. *X. fastidiosa* occurs in numerous strains which have only recently been well distinguished (Qin and others 2001). One strain causes citrus variegated chlorosis (CVC), a disease infecting citrus trees. Currently in Brazil about 5 million diseased trees are destroyed yearly, causing approximately $50 million in losses. Quarantines are in force in the United States to prevent introduction of the citrus strain. The regional strains of *X. fastidiosa* in forests and amenity shade trees of the North Central and Plains States do not appear to cause severe disease symptoms like those infecting grape and citrus.

Leaf scorch in trees can be caused by numerous unidentified abiotic causes as well as by the bacterial pathogen. A regional survey using detection of the bacterial pathogen provides a worthwhile evaluation of the proportion of scorch that can be attributed to the pathogen and the relative proportion attributable to environmental or unknown causes. This information is important to

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improving understanding of the causes of stress and decline in trees, particularly those stresses caused by unsuitable planting practices, and problems in urban soils and sites.

Having a measure of the incidence and distribution of BLS in the North Central and Plains States is worthwhile for establishing a baseline of current conditions. With a record of conditions, the influence of new or more effective vectors or of changes in climate warming can be documented more accurately. Because BLS is a factor in decline of trees, changes in distribution and incidence will impact forest health. If the changes are due to warming climate, decline in important forest species such as oaks and maples could be modeled and future trends in forest health could be predicted. Additionally, the host range of BLS in hardwoods and other woody plants is not yet well known, or known only for limited regions of the United States (McElrone and others 1999).

The planned objectives of this 2-year project included:

Objective 1: Determine the incidence of BLS in species of *Quercus, Acer, Platanus, Ulmus, Morus, Tilia* and other hardwoods in the 11 North Central and Plains States.

Objective 2: Determine the distribution of BLS in the 11 North Central and Plains States.

Objective 3: Relate the occurrence of BLS to mapped landscape-scale physiographic, edaphic, and climatic data.

METHODS

Organization of the project began with conference calls and a Web site initiated by a specialist[4] in the Forest Health Monitoring (FHM) Program of the Forest Service, U.S. Department of Agriculture. Conference calls included State foresters who had previously worked in forest health monitoring. Once procedures were agreed upon, the methods were posted on the Web site with description of the project and information on the etiology of the disease and illustrations of the symptoms. Additional volunteers were solicited by direct communication and by outreach and Extension articles. Volunteers included State Department of Agriculture employees, University Extension agents, State Department of Natural Resources employees, and private arborists, landscapers, and master gardeners. Cooperators and volunteers were advised to locate trees in their region of the 11 States showing leaf scorch during late July through October in 2008 and 2009. It was planned to sample urban and rural trees, and trees in forest stands. Sampling design was to sample from every symptomatic tree seen by the individual collector with a goal of obtaining 30 trees per year for each State by the total collectors in the State. Because of the scarcity of symptomatic trees, sampling was random for habitat, size, species and number. In some instances leaf scorch was evident by May,

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but samples were collected usually in August to allow the titer of the pathogenic bacteria to increase in the samples ("titer" refers to the detectable concentration of the pathogen in the host tissue). The sampling protocol was to collect two samples of scorched leaves per tree, old scorched leaves from one side of the tree and younger scorched leaves from the opposite side. Each sample was to include at least two to five leaves with symptoms attached to a shoot (approximately pencil width). After review of the number of samples and diversity of species collected in each State, the first year, successive year shoots and leaves were to be double-bagged in a self-addressed stamped Tyvek® envelope for shipment. Approximate tree locations were recorded by GPS coordinates or street addresses. Diameter at breast height was approximated or measured and percentage of crown showing scorch was recorded. Information was recorded on or in the envelopes and the exterior of the envelopes was pre-stamped with the categories of information requested from the collector. The requested information included: name and address of the collector, collection date, State, county, city, and street address location information, GPS coordinates indicating datum and format used, genus and species of the host, stem diameter size class (d.b.h.) estimated categories of sapling <5 inches (12.5 cm), pole 5-11 inches (12.5–27.5 cm), or large >11 inches (27.5 cm). Crown symptoms of percent of foliage affected by scorch symptoms, and percent dieback were recorded. For dieback, three approximated categories were used: low (<5 percent), moderate (5-20 percent), and high (>20 percent).

Envelopes were shipped the same day, or stored in a refrigerator at approximately 4 ˚C until shipment. Many leaf samples were photographed to record differences in scorch symptoms. Then, samples were processed to extract DNA from petiole xylem tissues. Quality of DNA, and presence and quantity of *X. fastidiosa* DNA was determined using machinery and reagents of the quantitative polymerase chain reaction DNA amplification methodology (real-time PCR or qPCR). Two standard protocols were used: the SYBR® Green protocol (Applied BioSystems; used in the Adams laboratory) or the TaqMan® protocol (Applied BioSystems; used in the Gould laboratory) with *X. fastidiosa* specific primers (Schaad and others 2002). For several samples, presence of *X. fastidiosa* also was determined with commercial enzyme linked immunosorbent assay (ELISA) technology (Agdia, Inc.) and also verified by qPCR. Three replicates of each sample were tested per assay. Detection sensitivity of qPCR methods ranged from 13.2 to 13,200,000 cells/mL. Quality assurance was checked and verified by sending a dozen or more samples between the two laboratories for comparison in double-blind assays (samples labeled and results matched to samples by a noninvolved student worker).

Positive and negative trees were mapped for distribution using MapSource® software (Garmin Ltd.), Google Maps®, and Google earth®. Climatic and physiographic isotherm lines were obtained from USDA Plant Hardiness zone maps. MapSource® distribution patterns were overlaid on black and white diagrams of

the Forest Service divisions, the North Central and Plains States, and the 1990 and 2006 hardiness zone border lines were superimposed individually and in combination on the final maps constructed in Adobe Illustrator®, and correlations examined and discussed.

RESULTS

During 2008 to 2009, approximately 471 trees were sampled that exhibited typical symptoms of leaf scorch. The volunteers collecting the samples were skilled at distinguishing scorch symptoms from insect damage, salt burn, nutrient deficiencies, anthracnose leaf diseases, leaf spots and other problems that can be confused with leaf scorch. Most of the host species collected were *Quercus* species, primarily *Q. rubra* with 89 samples submitted. Thirty samples of *Q. palustris*, *Q. macrocarpa*, and *Vaccinium corymbosum* were collected also. In total, 69 species of trees, shrubs and vines were ultimately submitted (table 10.1). A total of 106 collections of *Acer* spp. were also submitted. Many species of maple were collected in 2009 in Michigan following an unusual abiotic event where leaf scorch suddenly appeared in many trees in some counties without apparent relationship to weather or site conditions. The plant species that were determined to be infected with *X. fastidiosa* included 11 species, *Q. imbricaria*, *Q. macrocarpa*, *Q. palustris*, *Q. rubra*, *Q. bicolor* an unidentified *Quercus* sp., unidentified *Acer* sp., *Ulmus davidiana* var. *japonica*, *Morus rubra*, *Aesculus* sp., and *Fraxinus americana* 'Rosehill.' Fifteen collections of *Tilia* spp. were submitted but none were positive for BLS.

Table 10.1—Collections (2008–09) of plants with leaf scorch symptoms assayed by real-time qPCR

Species and cultivars	Number of samples	Positive assays[a]
Acer fremanii 'Autumn Blaze'	1	No
Acer ginnala (Amur maple)	8	No
Acer negundo (Box elder)	9	No
Acer platanoides (Norway maple, inc., 'Crimson King', 'Variagated')	21	No
Acer rubrum (Red maple)	10	No
Acer saccharinum (Silver maple)	3	No
Acer saccharum (Sugar maple)	16	No
Acer tataricum (Tatarian maple)	1	No
Acer sp. unidentified	37	Yes (2)
Aesculus sp.	1	Yes (1)
Aesculus sp. Hybrid	1	No
Aesculus x carnea (Red buckeye, 'Briotii')	1	No
Aesculus glabra (Ohio buckeye)	8	No
Aesculus hippocastanum (Horse chestnut)	4	No
Aesculus octardra (Yellow buckeye)	1	No
Amelanchier alnifolia (Serviceberry)	1	No
Caragana arborescens (Siberian peashrub)	1	No
Carpinus caroliniana (American hornbeam)	1	No
Catalpa speciosa (Northern catalpa)	1	No
Celtis occidentalis (Common hackberry)	1	No
Cercis occidentalis (Western redbud)	1	No
Gymnocladus dioicus (Kentucky coffee tree)	1	No
Fraxinus americana (White ash, inc. 'Rosehill')	4	Yes (2)
Fraxinus mandshurica (Manchurian ash)	3	No

continued

Table 10.1 (continued)—Collections (2008–09) of plants with leaf scorch symptoms assayed by real-time qPCR

Species and cultivars	Number of samples	Positive assays[a]
Fraxinus pennsylvanica (Green ash)	17	No
Juglans nigra (Black walnut)	1	No
Liquidambar styraciflua (American sweetgum)	1	No
Malus spp. (Flowering crabapple)	3	No
Malus domestica	1	No
Morus rubra (Mulberry)	4	Yes (1)
Parthenocissus quinquefolia (Virginia creeper)	1	No
Philliodendron amurense (Amur corktree)	1	No
Platanus x acerifolia (London planetree)	1	No
Platanus occidentalis (Sycamore)	1	No
Populus deltoids (Eastern cottonwood)	1	No
Populus sp. Hybrid	1	No
Populus tremula 'Erecta' (Columnar poplar)	2	No
Populus tremuloides (Quaking aspen)	3	No
Prunus serotina (Cherry)	1	No
Prunus virginiana (Chokecherry)	1	No
Pyrus sp. (Pear)	2	No
Pyrus ussuriensis 'Prairie gem'	4	No
Quercus alba (White oak)	25	No
Quercus acutissima (Sawtooth oak)	1	No
Quercus bicolor (Swamp white oak)	7	Yes (1)
Quercus coccinea (Scarlet oak)	1	No
Quercus ellipsoidalis (Northern pin oak)	1	No
Quercus imbricaria (Shingle oak)	3	Yes (1)

continued

Table 10.1 (continued)—Collections (2008–09) of plants with leaf scorch symptoms assayed by real-time qPCR

Species and cultivars	Number of samples	Positive assays[a]
Quercus macrocarpa (Bur oak)	30	Yes (2)
Quercus palustris (Pin oak)	30	Yes (8)
Quercus robur Hybrid (*Q. robur* 'Fastigiata' x *Q. bicolor* 'Regal Prince')	1	No
Quercus rober Hybrid (*Q. robur* x *Q. macrocarpa*)	1	No
Quercus rubra (Northern red oak)	89	Yes (2)
Quercus velutina (Black oak)	4	No
Quercus sp. unidentified	16	Yes (3)
Salix pentandra (Laurel leaf willow)	1	No
Sorbus aucuparia (European mountain-ash)	2	No
Syringa meyeri (Korean dwarf lilac)	1	No
Syringa reticulata (Japanese tree lilac)	3	No
Syringa villosa (Late or Villous lilac)	2	No
Syringa vulgaris (Common lilac)	3	No
Tilia americana (American linden, basswood)	12	No
Tilia cordata (Little-leaf linden)	3	No
Ulmus americana (American elm)	13	No
Ulmus davidiana var. *japonica* (Japanese elm)	4	Yes (1)
Ulmus parvifolia (Lacebark elm)	1	No
Vaccinium corymbosum (highbush blueberry)	29	No
Viburnum sp. (Viburnum)	1	No
Vitis sp. (Grape)	1	No

[a] 24 BLS (*Xylella fastidiosa*) positive assays, replicated three times, from 471 scorch samples, or 5 percent positive.

Sample collection in each State depended greatly on the interest and enthusiasm of volunteers. In 2008, the volunteers were primarily foresters in the Department of Natural Resources and tree enthusiasts in the Department of Agriculture in each State. In 2009, volunteers from the landscape industry, university extension service, and master gardeners also participated. Samples were received from 14 States. Four States were outside the North Central States and the Plains States, with one sample each from Colorado and Montana, two from Oklahoma, and nine from Utah. Of the 11 planned States, the following collections numbers were received: Illinois 18, Indiana 45, Iowa 0, Kansas 29, Michigan 143, Minnesota 18, Missouri 40, Nebraska 4, North Dakota 118, South Dakota 5, and Wisconsin 45. A remarkable diversity of species was collected in North Dakota. States that had BLS affected trees included Illinois, Indiana, Kansas, Michigan, Missouri (and Oklahoma). There were insufficient samples to verify whether BLS occurred in Iowa, Nebraska, and South Dakota. However, there is a record of BLS in Nebraska on mulberry (Sinclair and Lyon 2005). BLS-positive trees were not encountered in Minnesota or North Dakota. With 118 scorch samples from North Dakota, it is unlikely that BLS occurs there. The overall mean incidence of BLS-positive trees among 471 trees and woody shrubs exhibiting typical leaf scorch symptoms in late summer or fall was 5 percent (24 plants).

In figures 10.1 and 10.2, the distribution of the collected samples is illustrated by the dots within the boundaries of each State and the occurrence and location of BLS-positive trees are illustrated by the X markers within the States. The distribution of *X. fastidiosa* has been studied primarily in regards to occurrence

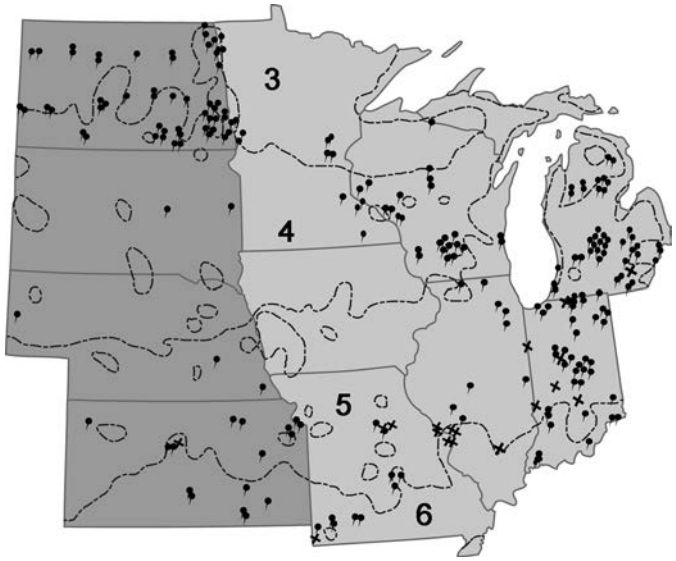

Figure 10.1—U.S. Department of Agriculture plant hardiness zone map for 1990, with dashed horizontal lines (isotherms) illustrating the boundaries of growth zones 3-7 of expected average annual minimum temperatures: Zone 3 = -35 to -40 ˚C, zone 4 = -29 to -35 ˚C, zone 5 = -23 to -29 ˚C, and zone 6 = -18 to -23 ˚C. Zones are constructed from records of lowest winter temperatures in the area in the preceding fifteen years (approximately). The locations of our scorch samples are represented as circular push pins for bacteria leaf scorch-negative trees and X-marks for BLS-positive trees. The geographical regions of the collections are shown, with Plain States (darker shading) and North Central States (lighter shading).

of Pierce's disease of grapes which is common throughout Southeastern North America and rare north of Tennesee (Anas and others 2008), although recently it has become a problem further north in Oklahoma (Smith and Dominiak-Olson 2009). BLS of hardwood trees has been commonly reported in Southern and mid-Atlantic States (Purcell and Hopkins 1996). Winter temperatures with 2-3 days below -12 ˚C reduce risk of Pierce's disease (Anas and others 2008) and are believed to be detrimental to survival of *X. fastidiosa* in hardwoods. High numbers of samples and zero to low numbers of BLS-positive trees in North Dakota (0-positive), Michigan (1-positive), and Wisconsin (2-positive), may be the result of unfavorable winter weather as the northernmost States show lower incidence of BLS than the States south of Michigan. Incidence does appear to increase for more Southern States, but sample numbers, experience of collectors, and number of collectors in the field, are undoubtedly interacting with data on determining frequency.

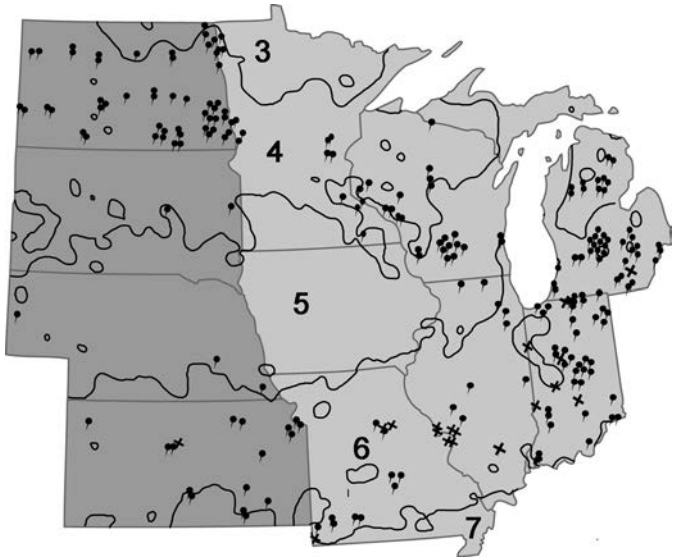

Figure 10.2—Newest U.S. Department of Agriculture plant hardiness zone map from 2006 with solid horizontal lines (isotherms) illustrating the boundaries of growth zones 3-7 of expected average annual minimum temperatures (zone 7 = -12 to -23 ˚C). Lowest winter temperatures are warming since the 1990 map; for example, the 1990 isotherms place the lower half of Michigan in zone 5, while the 2006 isotherms place the lower half in zone 6. This is a change of approximately 5 ˚C warmer. We hypothesize that bacteria leaf scorch (BLS) incidence will be increasing northward with the warming climate

Maps with isotherms, representing the mean lowest winter temperatures over 30 years, are not available for the States we were studying. However, a good determination of isotherms over 10-24 years is the Plant Hardiness Zones Map prepared for the U.S. Department of Agriculture (USDA). Each isotherm represents a 10 ˚F difference in the average annual minimum temperature. The isotherms from the current USDA map prepared in 2006 (The National Arbor Day Foundation 2006) are shown in figure 10.1 as solid lines and the 1990 Map isotherms as dashed lines both overlaid onto the occurrence and incidence markers for the collected plants of the 11 States. The BLS-positive trees occur in zone 6 (0 to -10 ˚F), except two trees from Wisconsin that occur in zone 5 (-10 to -20 ˚F). The warming of winter over the past decades is dramatically illustrated

by the differences between the solid and hatched lines from the two zone maps on the occurrence and incidence data. An additional worthwhile comparison to construct would be to overlay the ecoregions map (Vogel and others 2005) on the occurrence and incidence data.

DISCUSSION

The survey and research were relatively successful in developing a distribution and incidence database, and a host range, as baselines for future studies. As the diagnostic tests used for detecting BLS become more frequently and widely used by the national plant diagnostic network, then geographical outliers and an expanding host range should begin to accumulate. Our knowledge of the pathogen, the diseases it causes, and the symptoms expressed in woody plants should increase considerably as the detection technology advances.

In this study, 5 percent of trees showing scorched leaves were BLS-positive out of 471 samples and 69 species. Plant Hardiness Zone 5 is the northernmost limit for BLS in this study and Nebraska (positive report in Sinclair and Lyon [2005]) is included in zone 5, as well. Zone 6 is the region where 92 percent of BLS-positive samples originated in this study. Zone 7 (10 °F to 0 °F) includes the South Central States, such as Oklahoma, where *X. fastidiosa* (Pierce's disease) occurs in grapevines. We are certain that BLS is unlikely to be present in North Dakota (zones 4 and 3) at a titer sufficient for the qPCR detection, as no BLS-positive samples were found out of 118 trees exhibiting scorch. We assume this is due to the winter cold affecting either the vectors or the trees. We are not certain, due to sample size, whether BLS occurs in Minnesota where winter temperature may also exclude it. Minnesota has territory in three hardiness zones (zones 5, 4, and 3), the warmest being zone 5. Since Wisconsin has BLS-positive trees in zone 5, it is possible that the lower third of Minnesota also may have some BLS-positive trees. The new Plant Hardiness Zones Map (The National Arbor Day Foundation 2006) readily demonstrates the gradual warming of the continent over the past few decades and shows that the plants' northern ranges are extending. We hypothesize that this extension is, and will be, effecting the distribution and incidence of BLS. Additionally, the incidence and distribution of BLS might be affected by the local variations of moisture, soil type, microclimates, winds, and other conditions affecting plant growth and health.

Improvement in the methodology for detection of *Xylella* is needed for trees with low titers of the bacterium, particularly trees in the northern States. Collections during 2008-2009 found two red oak trees in Wisconsin that were BLS-positive, however, detection was erratic sometimes giving positive assays and other times negative assays. Double-blind tests were conducted with Rutgers University on these samples using petioles (unprocessed) and DNA (processed) samples so that precision in extraction methods and assay sensitivity could independently be compared. The double-blind tests revealed that the samples (unprocessed or processed) had titers at the limit of detection by

the current technology. The limit of detection is at approximately one cell of the pathogen in the volume of processed extraction applied to the assay. The results revealed that the quality control was successful. The results also uncovered the possibility that the current assays may be failing to detect infections in the northern regions due to problems of low titer. One hypothesis needing testing is that many northern hardwoods may harbor the pathogen at titers below current detection. If this is the case, then increasing incidence of BLS with increasingly warm climate will be due to a buildup of titer, rather than advancing movement of vectors.

To improve our understanding of the epidemiology of BLS, more accurate detection methods should be developed. Because qPCR detection methods are already as sensitive as one cell per sample, increasing sensitivity by concentrating samples to increase pathogen cell numbers is a reasonable approach. Higher titers may exist in roots in northern climates and further research is warranted to verify this issue. Processing larger samples would yield more target for the pathogen but also increase competing host material which may or may not inhibit or mask detection. Two approaches to this potential problem would be separating pathogen target from host material or selectively increasing pathogen target while decreasing or subtracting host material. Processing greater masses of petiole tissue can be readily accomplished with reasonable numbers of samples. To separate pathogen target DNA from host DNA, pulse field gel electrophoresis (PFGE) (Qin and others 2001) or cesium chloride density centrifugation (Tran-Nguyen and Gibb 2007) have been successfully employed. A method of increasing the pathogen target while decreasing the amount of host competing target known as suppression subtraction hybridization (SSH) (Cimerman and others 2006) has been developed and successfully employed in similar pathogen-host extractions.

Diagnosis and detection by ELISA is quick and relatively sensitive, and development of qPCR has improved sensitivity tenfold. However, to economically process samples for qPCR assays, numerous (10 to 20) samples need to be loaded on the machine at one time. Waiting for sufficient samples to be received, however, delays diagnosis, which aggravates cooperators and discourages public participation. The solution is to combine the two assays, utilizing the less sensitive ELISA technique for expediting diagnosis and utilizing the more sensitive qPCR technique for increased accuracy.

CONCLUSIONS

In this study, 5 percent of trees showing scorched leaves were BLS-positive out of 471 samples and 69 species. Plant Hardiness Zone 5 is the northernmost isotherm for BLS in this study, and Nebraska [positive report in Sinclair and Lyon 2005)] is included in zone 5, as well. The isotherm lines delimiting zone 6 encompassed 92 percent of BLS-positive samples. Zone 7 (10 ˚F to 0 ˚F) includes the southern central States, such as Oklahoma, where *X. fastidiosa* occurs in grapevines.

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