Distribution of *Xylella fastidiosa* in Oaks in Florida and Its Association with Growth Decline in *Quercus laevis*

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ABSTRACT

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A survey of more than 200 trees has documented the widespread occurrence of *Xylella fastidiosa* in Florida oak populations. The pathogen was detected readily via enzyme-linked immunosorbent assay in oaks exhibiting decline or leaf scorch symptoms and was infrequently detected in asymptomatic trees. It was also associated with reduced growth in *Quercus laevis* as measured by current-year shoot length. The occurrence of *X. fastidiosa* in *Q. laevis* and the evidence for its occurrence in *Q. incana* represent first reports for these oak hosts. The role of *X. fastidiosa* in oak decline scenarios deserves further attention.

Additional keywords: leaf scorch, oak decline, *Quercus* spp.

The fastidious xylem-inhabiting bacterium, *Xylella fastidiosa* Wells et al. (24), is associated with and in many cases causes leaf scorch and decline diseases in a variety of plant species (11,12,17,24). Many leaf scorch disorders in broad-leaved tree species are associated with or attributed to *X. fastidiosa*, and the organism is widespread in tree populations (1–13,15–17,19,20,24). *X. fastidiosa* has been known to occur in trees in Florida for years (3,11,12), and has recently been reported in association with decline in live oak (*Quercus virginiana* Mill.) in southwest Florida (15). In 1992 and 1993, we evaluated more than 200 oaks throughout Florida for the presence of *X. fastidiosa*. Our objectives were (i) to determine the distribution of the pathogen in the state's oak populations, and (ii) to ascertain whether the bacterium was present (and common) in apparently healthy oaks as well as in oaks exhibiting leaf scorch, decline symptoms, or both.

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MATERIALS AND METHODS

Sample tree identification. In August 1992, County Foresters with the Florida Division of Forestry were asked to locate individual oaks representative of locally prevalent species in natural or urbanized environs which exhibited decline symptoms, including 25 to 30% crown dieback, premature leaf abscission, small, sparse, chlorotic foliage, definitive leaf scorch, or a combination of symptoms. At the same time, they were asked to locate nearby apparently healthy, asymptomatic individuals of the same species to be included in sample pairs. These sample pairs were visited in October to December 1992, and if suitable (free from obvious insect, disease or mechanical injury, site disturbance, chemical influences, etc.) were sampled for detection of *X. fastidiosa*. Twenty of the trees sampled in 1992 were sampled again in the fall of 1993.

Sampling and processing for detection of *X. fastidiosa.* Four to six small branchlets, approximately 0.25 to 0.75 cm in diameter, with several buds and attached leaves, were removed from the crowns of sample trees with a pole pruner. Leaves on sample branchlets from apparently healthy trees exhibited no symptoms of disease. Those on sample branchlets from declining or leaf-scorched trees may or may not have exhibited symptoms, depending upon the locus of individual sample branchlets within the crowns of symptomatic trees. In most cases, branchlets with symptomatic leaves were selected. Sample branchlets were placed in labeled plastic bags and transported in an ice chest to the Florida Division of Plant Industry's Plant Pathology Laboratory in Gainesville.

In the laboratory, several 1.0- to 1.5-cm segments of twig tips with terminal bud clusters were cut from sample branchlets. Subsamples of these twig tips were combined with short segments of leaf petioles or midribs from the leaves of the same tree to provide a composite tissue sample of approximately 1.0 to 1.5 g (fresh weight). Composite tissue samples were then squeezed in a Model 1 Leaf Squeezer (Ravenel Specialties Co., Seneca, SC), diluted 1:10 wt/vol in extraction buffer (20), and subjected to double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) with commercial polyclonal antisera (Agdia, Inc., Elkhart, IN) using peroxidase-labeled conjugate. Positive and negative controls were included with each lot of samples processed. Samples were considered positive for *X. fastidiosa* when they produced a visually intense yellow reaction as compared to negative controls, and when absorbance measured at 490 nm with an Emax precision microplate reader (Molecular Devices Corp., Sunnyvale, CA) exceeded 2.5× that of the negative controls. Following processing at the Plant Pathology Laboratory, residual sample branchlets and leaves from approximately 25% of our sample trees were immediately forwarded to the University of Florida's Central Florida Research and Education Center in Leesburg for verification. There, these samples were again processed via ELISA (above) and isolations for *X. fastidiosa* were attempted on periwinkle wilt medium (6).

Assessing growth decline in *Quercus laevis*. Because early field observations suggested a pronounced reduction in current-year (1992) shoot elongation in turkey oaks (*Q. laevis* Walt.) exhibiting symptoms of decline or abundant leaf scorch, measurements were made to verify this phenomenon. Four branches were removed from random locations within the crowns of 16 apparently healthy trees and 16 trees exhibiting definitive leaf scorch. Sample tree pairs were distributed among four counties (Clay, Madison, Suwannee, and Citrus) in northern Florida. In the laboratory, current-year growth on each of the first 10 shoots on each branch, beginning with the terminal shoot and proceeding in a basipetal direction, was measured to the nearest 0.5 cm. Differences between mean shoot lengths for symptomatic versus asymptomatic and paired *Xylella*-positive versus *Xylella*-negative trees were evaluated for significance at $P = 0.05$ and $P = 0.01$ using standard two-tailed t tests (22).

RESULTS

A total of 206 oaks were sampled and processed in 1992. Species represented in the sample included turkey oak, southern red oak (*Q. falcata* Michx.), live oak, laurel oak (*Q. laurifolia* Michx.), water oak (*Q. nigra* L.), post oak (*Q. stellata* Wangenh.), bluejack oak (*Q. incana* Bartr.), and swamp chestnut oak (*Q. michauxii* Nutt.; Table 1). Four species (*Q. laevis*, *Q. falcata*, *Q. virginiana*, and *Q. laurifolia*) were sampled extensively whereas four others (*Q. nigra*, *Q. stellata*, *Q. incana*, and *Q. michauxii*) were sampled infrequently, due primarily to their relative scarcity in the landscape and the relative lack of decline or leaf scorch symptoms occurring on them. Overall, very few healthy, asymptomatic oaks gave positive ELISA responses for *X. fastidiosa*. In contrast, high percentages of oaks with decline, leaf scorch symptoms, or both reacted positively for the pathogen. This association was particularly evident in *Q. laevis* and *Q. falcata*, but less so in *Q. virginiana* and *Q. laurifolia* (Table 1).

Verification processing of residual sample materials at the University of Florida's Central Florida Research and Education facility showed an excellent correspondence to results obtained via ELISA at the Plant Pathology Laboratory. Repeat ELISA results were nearly a 1:1 match with preliminary ELISA readings, and *X. fastidiosa* was isolated from nearly half of the samples (predominantly turkey oak) yielding a positive reaction for the pathogen via ELISA (*data not shown*).

Shoot measurements on *Q. laevis* revealed significant differences in current-year (1992) shoot growth between leaf-scorched trees and asymptomatic trees, as well as between paired *Xylella*-positive and *Xylella*-negative trees. Current-year shoots on trees with leaf scorch symptoms were approximately 29% shorter than those on asymptomatic trees, and shoots on *Xylella*-positive trees were approximately 38% shorter than those on paired *Xylella*-negative trees (Fig. 1).

Eighteen turkey oaks and 2 bluejack oaks from the 1992 sample were sampled again in 1993 (Table 2). Of the 20 oaks sampled in 1993, 15 showed no change from their 1992 condition with respect to the presence of *X. fastidiosa*. Four oaks, however, showed a change from *Xylella*-free to *Xylella*-positive over the course of the year. Only 1 oak reflected a change from *Xylella*-positive to *Xylella*-negative (Table 2).

Figure 2 displays the geographical distribution of oaks sampled in Florida and the known distribution of *X. fastidiosa* within the state's oak populations.

DISCUSSION

In our ELISA analyses, we deliberately took a conservative approach toward identifying *Xylella*-infected trees. Accordingly, only those samples that gave clear and strong positive reactions were counted as *Xylella*-positive. This approach strengthens confidence in our positive data, but in actuality may have contributed to an overall underestimate of the pathogen's occurrence and distribution. Such an approach may have also contributed to the very limited number of *Xylella*-positives we recorded for symptomatic *Q. virginiana* and *Q. laurifolia*. These species are known hosts of *X. fastidiosa* and symptoms are often coincident with, if not indicative of, the presence of the organism (12,15). Several samples from symptomatic and asymptomatic individuals of these two species (especially *Q. virginiana*) gave variable but unconvincing "positive" reactions; if we erred in our calls, we erred on the side of conservatism.

Many investigators have reported the occurrence of *X. fastidiosa* in symptomatic oaks only (4,7,8,15). Others have noted the failure to detect the pathogen or its anti-

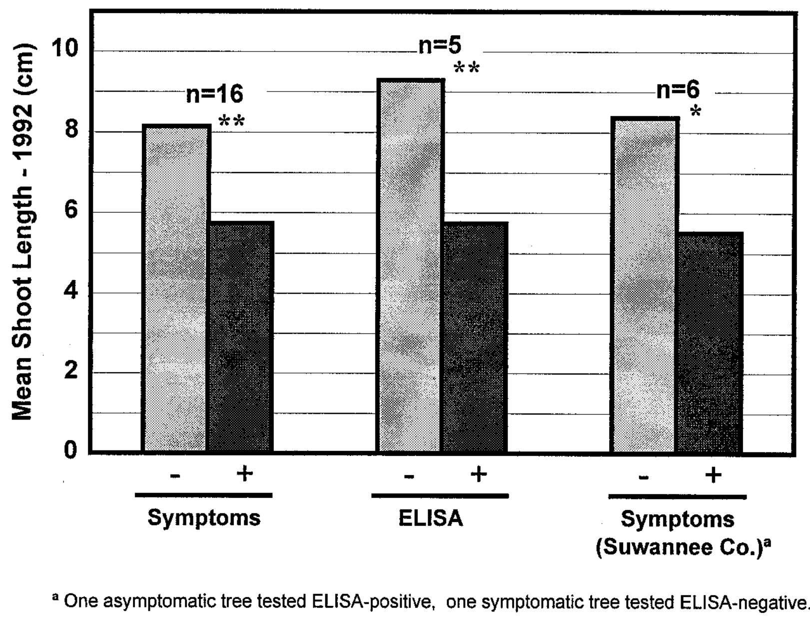

Fig. 1. Current-year (1992) shoot growth of *Quercus laevis* with and without definitive leaf scorch symptoms and positive enzyme-linked immunosorbent assay reactions for *Xylella fastidiosa*. Column pairs annotated with double or single asterisks indicate significant differences at $P = 0.01$ and 0.05, respectively.

Table 1. Oaks sampled in Florida during 1992 and results of enzyme-linked immunosorbent assay (ELISA) screening for *Xylella fastidiosa*

	No. trees sampled by crown condition			%trees ELISA-positive		
Species	Healthy[a]	Symptomatic[b]	With leaf scorch	Healthy[a]	Symptomatic[b]	With leaf scorch
Quercus laevis	42	46	42	2	65	60
Q. falcata	9	11	8	0	46	63
Q. virginiana	24	22	1	0	9	0
Q. laurifolia	16	19	2	0	5	0
Q. nigra	2	4	0	50	50	…
Q. stellata	1	6	4	0	0	0
Q. incana	1	2	1	0	100	100
Q. michauxii	0	1	1	0	0	0
Total: weighted $\bar{x}$	95	111	59	2	38	53

[a] Apparently healthy; no visible crown or foliar symptoms.
[b] Including dieback, crown-thinning, defoliation, chlorosis, and leaf scorch.

gen(s) in symptomless oaks (5,9,20). In our investigation, we found evidence of the organism in both symptomatic and asymptomatic trees, albeit extremely limited in the latter (Table 1 and 2). Presumably, the sensitivity of the ELISA methodology, the large numbers of oaks we sampled, and the possibility of latent disease development could account for this presence in asymptomatic trees.

A seemingly disproportionate number of symptomatic or leaf-scorched survey trees from the southern half of peninsular Florida were *Xylella*-negative when subjected to ELISA. Based on the apparently strong association between *X. fastidiosa* and decline or leaf scorch symptoms (Table 1 and 2), this was unexpected. While several explanations could be posited for this phenomenon (e.g., processing error, absence of pathogen), we suspect season or timing of sampling could be a factor. McGovern and Hopkins (15) have reported *X. fastidiosa* from symptomatic oaks at these latitudes (data reflected in southwest Florida; Fig. 2). They sampled primarily in early October, whereas most of our south-Florida samples were collected later in the year. However, as in the survey reported here, McGovern and Hopkins (*unpublished results*) also failed to confirm *X. fastidiosa* in symptomatic oaks in Broward County in southeastern peninsular Florida.

Overall, results of our survey provide evidence that *X. fastidiosa* is widespread in Florida oak populations, strongly associated with leaf scorch symptoms, and apparently related to, if not a cause of, growth decline in *Q. laevis*. These results support and expand the more limited reports of Hopkins and Alderz (12) and McGovern and Hopkins (15). In addition, this paper provides the first report of occurrence of this pathogen in *Q. laevis* and preliminary evidence of its occurrence in *Q. incana* (not verified in this species by isolation or direct observation).

The prevalence of *X. fastidiosa* in oaks in Florida in both natural and urbanized environs, especially in the very abundant *Q. laevis*, suggests that host oak species may represent a significant inoculum reservoir for the pathogen. Further, the coincidence of *Xylella*-associated leaf scorch, oak decline, and their overlapping symptomatologies in Florida and the southern United States (4,8,9,12,14,21,23) raises legitimate questions regarding the possible role or roles of *X. fastidiosa* in regionwide oak decline. Also, in Florida at least, growth decline and *X. fastidiosa* in *Q. laevis* should be evaluated in relationship to root rot caused by *Armillaria tabescens* (Scop.) Dennis, Orton & Hora, a common disease of this oak species (Barnard, *unpublished results;* 18).

Table 2. Tree crown conditions and associated enzyme-linked immunosorbent assay (ELISA) test results for *Xylella fastidiosa* in oaks sampled in 1992 and 1993

		Crown condition/symptoms[a]		ELISA response	
Species, location	**1992 tree no.**	**1992**	**1993**	**1992**	**1993**
Quercus laevis					
Clay Co.	47	LS	LS	+	+
	48	H	H	–	+
	49	LS	LS	+	+
	50	H	H	–	+
	51	H	H	–	–
	52	LS	LS	+	+
Suwannee Co.	53	H	H	–	–
	54	LS,CT	LS,CT,DB	+	+
	55	LS	LS	+	+
	56	H	H	–	+
Madison Co.	61	H	H	–	–
	62	LS	LS	+	+
Citrus Co.	63	LS,DEF,DB	LS	+	–
	64	H	H	–	–
	65	H	H	–	–
	66	LS,DB	LS,CT,DB	+	+
	67	LS	LS	+	+
	68	H	LS	–	+
Q. incana					
Madison Co.	57	LS,CT	LS,CT,DEF,DB	+	+
	58	H	H	–	–

[a] Visual assessment of crown/foliage symptoms: H = healthy, LS = leaf scorch, CT = crown thinning, DEF = defoliating, DB = dieback.

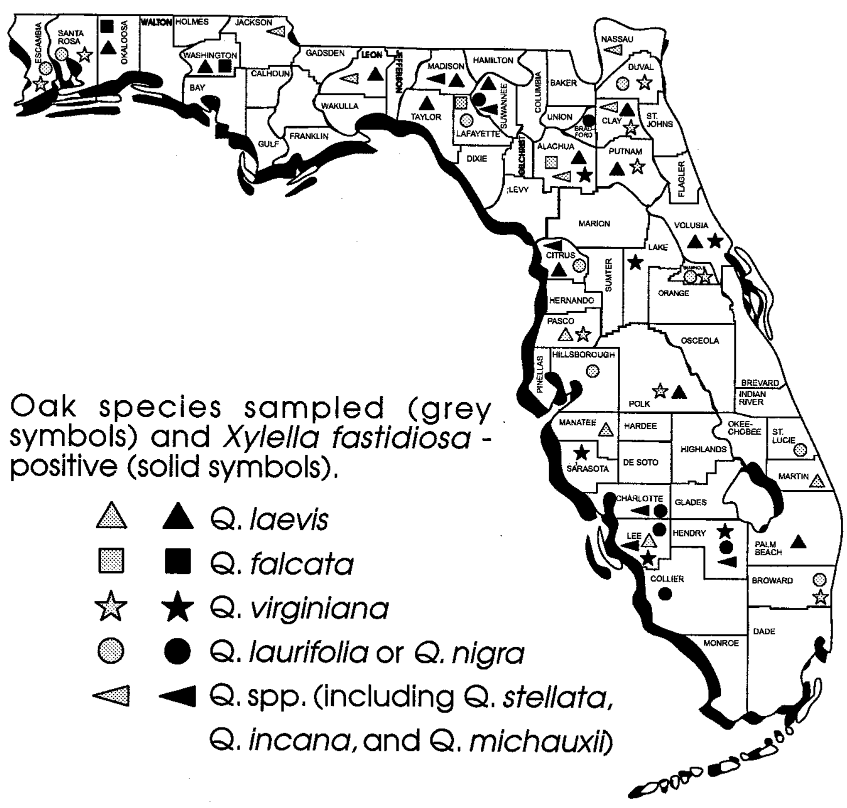

Fig. 2. Distribution of *Quercus* spp. sampled for *Xylella fastidiosa* in Florida and known distribution of the pathogen in the state's oak populations. Map reflects survey data and incidental data accumulated by authors over time.

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