

An extended range for oak wilt and *Ceratocystis fagacearum* compatibility types in the United States

D. N. APPEL, C. FROST DREES, AND J. JOHNSON

Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, and Texas Agricultural Extension Service, Texas A&M University, College Station, TX, U.S.A. 77843

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Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has been found in 31 Texas counties, significantly extending the southern range of the disease in the United States. The 101 isolates obtained from 76 diseased oaks in 22 of those counties were assayed for sexual compatibility using a paired plug or spermatizing technique. There were 51 type A isolates and 44 type B isolates; 6 of the isolates could not be typed. A majority of multiple-tree infection centers contained only one compatibility type and only one diseased tree yielded both compatibility types. The distribution of compatibility types and behavior of *C. fagacearum* in Texas are consistent with those observed in other states.

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Les auteurs ont repéré le *Ceratocystis fagacearum* (Bretz) Hunt, l'agent causal de la flétrissure du chêne, dans 31 comtés du Texas, ce qui étend l'aire de distribution de la maladie vers le sud, de façon significative. Les 101 isolats obtenus à partir de 76 chênes malades dans 22 de ces comtés ont été évalués pour leur compatibilité sexuelle en utilisant une technique de spermatisation ou couplage d'inoculum. Le type A a été retrouvé 51 fois et le type B 44 fois; le type de 6 souches est resté indéterminable. Une majorité de centres d'infection impliquant plusieurs arbres ne contenait qu'un seul type de compatibilité, et un seul des arbres malades portait les deux types de compatibilité à la fois. La distribution des types de compatibilité et le comportement du *C. fagacearum* au Texas ne diffèrent pas de ce qui a été observé dans les autres états américains.

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Introduction

The 1961 discovery of *Ceratocystis fagacearum* (Bretz) Hunt in Dallas, TX (6), extended the known oak wilt distribution 200 mi (1 mi = 1.609 km) to the south of previously reported occurrences, but was subsequently regarded as an anomaly in the range of the disease (15). The southern spread of *C. fagacearum* was believed to be inhibited by high temperatures and a lack of fungal mat formation resulting from competitive colonization of diseased trees by *Hypoxylon* spp. (14). For the most part, the reported distribution of oak wilt remained unchanged from 1951 to 1980 (9). This range extended from the upper Mississippi River valley in Minnesota and Wisconsin eastward to Pennsylvania, south to South Carolina, and west to Oklahoma. Disease severity was greatest within the northwest part of that range (9).

In recent years, *C. fagacearum* has been isolated from dying oaks at several locations in central Texas (1,2,11). Localized epidemics have resulted in severe losses of live oaks (*Quercus virginiana* Mill. and *Quercus fusiformis* Small) and Spanish oaks (*Quercus texana* Buckl). Both sexual compatibility types (A and B) of the self sterile, hermaphroditic fungus were identified (1), but the frequency of each throughout the oak wilt range in Texas was not described. This paper describes the county distribution of oak wilt in Texas and distribution of *C. fagacearum* compatibility types within the state.

Materials and methods

Sources of cultures and culture methods

Isolations from 58 live oaks, 16 Spanish oaks, 1 post oak (*Quercus stellata* Wangh.), and 1 blackjack oak (*Quercus marilandica* Muenchh.) exhibiting typical oak wilt symptoms yielded 101 isolates of *C. fagacearum*. These isolates were obtained from diseased trees in 22 Texas counties located with the assistance of county extension personnel. Oak mortality was also located during a systematic survey of selected areas in central Texas using aerial color-infrared photography (2). Branch and bole samples were removed from symptomatic

TABLE 1. Constituents of a modified maltose – casein hydrolysate medium for production of perithecia by paired isolates of *Ceratocystis fagacearum*

Constituent	Quantity
Maltose	5.0 g
Casein hydrolysate	1.0 g
Yeast extract	2.0 g
KH ₂ PO ₄	0.5 g
MgSO ₄ · 7H ₂ O	0.5 g
ZnSO ₄ · 7H ₂ O	0.2 mg
FeSO ₄ · 7H ₂ O	0.2 mg
MnSO ₄ · 7H ₂ O	0.1 mg
D-Biotin	5.0 mg
Thiamine HCl	1.0 mg
Agar	20.0 g
Distilled, deionized H ₂ O	1.0 L

trees, stored on ice, and returned to the laboratory for analysis. In the laboratory, all bark tissues were removed from the samples, and sapwood chips, approximately 1.0 × 1.0 × 0.25 cm, were placed on potato-dextrose agar (PDA) prepared from fresh potatoes and poured in 9 cm plastic petri plates. The plates were stored at room temperature for 2–4 weeks and observed for the appearance of *C. fagacearum*. When the pathogen was successfully isolated, cultures were transferred to PDA plates, single spored, and stored in 10 mm screw-cap test tubes until assayed for sexual compatibility.

Compatibility determination

Sexual compatibility was determined using either a paired plug or a spermatization technique. All cultures were incubated in the dark at 25°C on a maltose–casein hydrolysate (MCH) medium similar to that developed by Barnett (3) and recommended by others (5,10) (Table 1). The pH of the prepared agar was 5.72.

Cultures of known compatibility type were obtained from Dr. W. L. MacDonald, Department of Plant Pathology and Agricultural Microbiology, West Virginia University, Morgantown, WV, U.S.A.

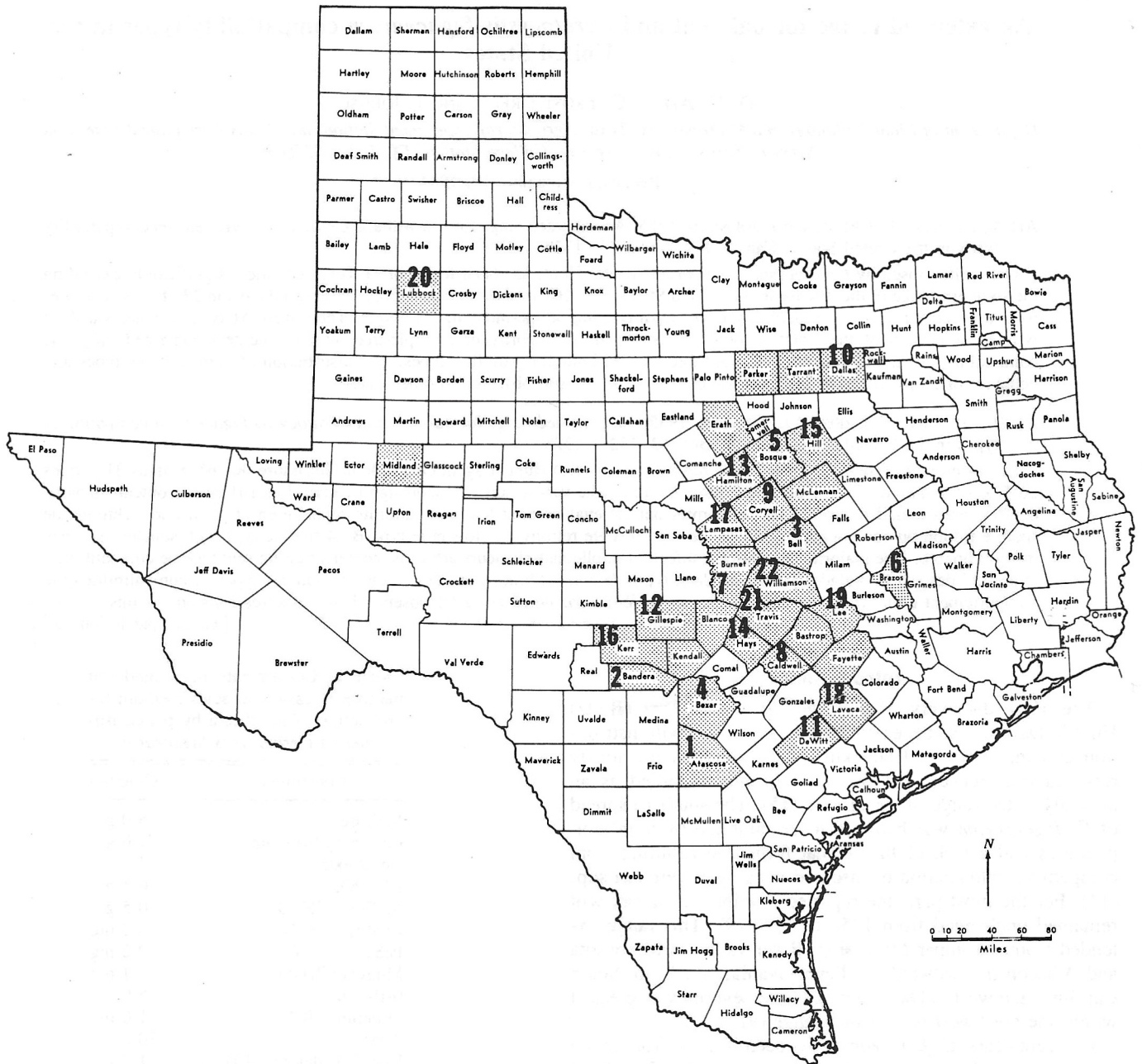


FIG. 1. County distribution of oak wilt in Texas. Shading indicates the presence of oak wilt in a county. Numbered counties correspond to those in Table 2.

26505. These isolates were from Arkansas, West Virginia, and Wisconsin. With the paired-plug technique for spermatization, cultures of unknown type were grown at 25°C on PDA. After 10 days, 5-mm plugs were removed and paired with both A and B isolates on 15 × 60 mm petri plates containing the MCH medium. Three replications of each cross were made (three A's and three B's). Isolates were grown alone to serve as checks.

Spermatization was also accomplished using a method described by Kaufman (10). Known A and B isolates were grown for 10 days at 25°C in the dark on 15 × 60 mm petri plates containing MCH medium. Droplets of sterile distilled water were placed on the known cultures and agar plugs of the test isolates were rubbed across the surface to spread conidia over receptive hyphae. Two replications of each cross were made. Using either technique, production of fertile perithecia indicated a positive cross. Isolates producing fertile perithecia when crossed with a known A were typed B and vice versa. Crosses failing to produce ascospores with the first attempt were repeated.

Results

County distribution

Oak wilt has now been identified in 31 Texas counties (Fig. 1). Most of these counties lie within the Edwards Plateau and Cross Timbers regions of central Texas. The most severe outbreaks of oak wilt were observed in the southwestern portion of the range depicted in Fig. 1.

Sexual compatibility

Fertile perithecia usually appeared within 5 days following spermatization or merging of cultures in the paired plug tests. There were 51 type A and 44 type B isolates among those obtained from the 22 counties (Table 2). Six of the isolates could not be typed. None of the untreated, single spore cultures produced fertile perithecia.

Both compatibility types were evenly distributed throughout

TABLE 2. The occurrence of *Ceratocystis fagacearum* compatibility types in 22 Texas counties

County ^a	No. of host trees	Compatibility type		
		A	B	Untyped
1. Atascosa	1	3	0	—
2. Bandera	7	3	3	1
3. Bell	3	5	0	1
4. Bexar	2	2	0	—
5. Bosque	1	0	1	—
6. Brazos	1	1	0	—
7. Burnet	8	10	5	—
8. Caldwell	1	3	0	—
9. Coryell	1	1	2	—
10. Dallas	1	0	1	—
11. DeWitt	1	0	1	—
12. Gillespie	5	1	5	1
13. Hamilton	1	0	0	2
14. Hays	1	0	1	—
15. Hill	1	1	0	—
16. Kerr	24	12	13	—
17. Lampasas	5	3	2	—
18. Lavaca	2	2	0	1
19. Lee	1	0	1	—
20. Lubbock	2	2	1	—
21. Travis	5	2	3	—
22. Williamson	2	0	5	—
Total	76	51	44	6

^aNumber for each county refers to the numbered position shown in Fig. 1.

the range of *C. fagacearum* in Texas. In Kerr County, where 24 trees were sampled, similar numbers of A and B isolates colonized the trees (Table 2). Multiple isolates were assayed from 12 trees throughout the 22 counties, but only 1, a Spanish oak in Coryell County, yielded isolates of both compatibility types.

Ten distinct, individual oak wilt centers were sampled with two or more trees and both compatibility types were found in only two (Table 3). Only one of the two compatibility types was isolated from the remaining eight oak wilt centers.

Discussion

The state-wide ratio of the two *C. fagacearum* compatibility types in Texas is similar to ratios described in other states. Isolates of both A and B types are most often found in equal numbers in areas affected by oak wilt (7, 12, 16). In most Texas counties where more than one tree was sampled, both types were found. Also, only one diseased tree yielded both an A and B isolate among the 76 trees sampled in Texas. A mixed isolate (one culture containing both compatibility types) was never isolated from any tree. Mixed isolates and the separate isolation of a type A and type B isolate from a single tree are seldom found in other states (4). A majority of the discrete, multiple-tree infection centers contained only one compatibility type (Table 3). Kaufman (10) found the same was true of infection centers in West Virginia and suggested such an occurrence might be due to root-graft transmission of a single compatibility type within a center. The tendency for live oak to produce root sprouts and maintain common root systems in Texas probably results in a majority of discrete, multiple-tree infection centers containing only one compatibility type.

The occurrence of both *C. fagacearum* compatibility types in close proximity is important to the survival and spread of the pathogen. Fungal mats with fertile perithecia have been found

TABLE 3. Compatibility types of *Ceratocystis fagacearum* isolates found within 10 distinct oak wilt centers in central Texas

County	No. of host trees	Compatibility type		
		A	B	Untyped
Bandera	2	1	1	—
Bexar	2	2	0	—
Burnet	8	10	5	—
Kerr	2	0	2	—
Kerr	2	2	0	—
Lampasas	2	0	2	—
Lampasas	2	2	0	—
Lavaca	2	1	0	1
Williamson	5	0	5	—

in Kerr and Burnet counties. This potential for sexual recombination increases the variability and adaptability of the fungus and provides ascospores as additional inoculum for overland spread.

The occurrence of oak wilt in Midland and Lubbock counties is unusual because each of these locations is 240–280 km west of any natural tree regions in the state. The fungus was isolated from *Q. virginiana* in Midland and *Q. texana* in Lubbock. Each of these species is a commonly planted ornamental at those locations. The mechanism for introduction of *C. fagacearum* into planted trees in these counties is unknown. The remainder of the range of *C. fagacearum* in Texas largely coincides with the ranges of *Q. virginiana* and *Q. fusiformis* (8, 13). An important exception is the lack of any known oak wilt in those counties nearest to the Gulf of Mexico in southeast Texas. The potential for spread of *C. fagacearum* into these counties and other Gulf States with native live oak is unknown.

These results significantly extend the southern range for *C. fagacearum* in North America. Texas isolates of *C. fagacearum* are heterothallic, self-sterile, and are sexually compatible with isolates from Arkansas, Wisconsin, and West Virginia. Distributions of compatibility types were similar to distributions previously reported. Although the fungus is found colonizing new hosts and is growing under new environmental conditions, the behavior of *C. fagacearum* in Texas is consistent with that observed in other parts of the United States. The abundance of oak wilt on *Q. virginiana* in central Texas justifies a further survey for *C. fagacearum* on the numerous semi-evergreen oaks found in west Texas and northern Mexico.

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