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TREE SUSCEPTIBILITY, INOCULUM AVAILABILITY, AND POTENTIAL VECTORS IN A TEXAS OAK WILT CENTER

by D.N. Appel, R. Peters[1], R. Lewis, Jr[2].

Abstract. Three factors important to the spread of *Ceratocystis fagacearum,* the oak wilt pathogen, were studied in central Texas during 1983-1985. These factors were: 1) the formation of fungal mats on diseased Spanish oaks (*Quercus texana*), 2) the activities of nitidulid beetles, and 3) the seasonal susceptibility of live oak (*Q. fusiformis*) to infection by the pathogen. Fungal mats formed on 73% and 31% of the trees observed in March of 1984 and 1985, respectively. Peaks of nitidulid activity were observed during February - May of each year. Inoculations were most successful when made in roots or main stems during March or late May. These observations indicate that inoculations by contaminated nitidulids are most likely to occur in spring. Wounding should be avoided then to minimize insect transmission of the fungus.

Résumé. Trois facteurs importants à l'expansion du *Ceratocystis fagacearum*, le pathogène responsable de la flétrissure du chêne, furent étudiés dans les régions centrales du Texas de 1983 à 1985. Ces facteurs étaient: 1) la formation de tissus fongiques sur les chênes espagnols (*Quercus texana*), 2) les activités d'insectes (nitidulidés), et 3) la susceptibilité saisonnière du chêne vert (*Quercus fusiformis*) à l'infection par le pathogène. Le tissu fongique s'est formé sur 73% et 31% des arbres observés en mars 1984 et 1985, respectivement. Une activité maximale des insectes fut observée entre le mois de février et de mai de chaque année. Les inoculations furent mieux réussies lorsqu'elles furent réalisées dans les racines ou sur la tige principale en mars ou à la fin mai. Ces observations indiquent que les inoculations par les insectes contaminés sont plus susceptibles d'arriver au printemps. Les blessures devraient être évitées afin de minimiser la transmission du pathogène par les insectes.

Fresh wounds on oaks are important infection courts for the vascular wilt fungus, *Ceratocystis fagacearum.* When trees near centers of oak wilt are wounded, the likelihood of infection increases over that of unwounded trees (4, 11, 12, 14). Wounded oaks are most susceptible to infection during the spring, usually May-June (9, 11). Sap-feeding nitidulid beetles (Coleoptera: nitidulidae) will transmit *C. fagacearum* when they are contaminated with spores and caged over wounds on healthy oaks (6, 16). In nature, these beetles acquire spores while feeding and breeding on fungal mats formed beneath the bark of diseased red oaks (*Quercus* sub-genus Erythrobalanus) (20). This evidence leads to the recommendation of avoiding wounding in the spring to reduce the opportunity for insect dispersal of the fungus (7, 8).

The successes of oak wilt control efforts vary throughout the disease range (20). For example, fungal mat formation and transmission by nitidulids are considered inconsequential in some states because mats are rarely found (3, 9). For this reason, all stages in the disease cycle on live oaks in Texas are being studied before endorsing control methods developed elsewhere. Live oaks (*Q. fusiformis, Q. virginiana*) are the primary hosts for *C. fagacearum* in Texas, and differ ecologically, physiologically, and anatomically from deciduous red oaks and white oaks (sub-genus Leucobalanus) (1, 13, 15). Spanish oaks (*Q. texana*) are severely affected by oak wilt in Texas and are known to support fungal mat formation, but comprise a relatively small component of the oak

1. Present Address: Bartlett Tree Experts, 6061 Walzem Rd., San Antonio, 78239
2. Present Address: Northeastern Forest Experiment Station, 370 Reed Road, Broomall, PA 19008
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savanahs where wilt is epidemic (21). Also, central Texas experiences higher summertime temperatures and milder winters than those areas where control measures were originally developed. This study examined the potential for inoculum formation, seasonal host susceptibility, and the activities of suspected vectors in Texas.

Materials and Methods

Study Site. These experiments involved a large conglomerate of oak wilt infection centers located 20 km north of Burnet, TX, (233 km northwest of College Station, TX). The area of tree mortality occupied approximately 112 ha. This typical central Texas site consisted of a large, nearly homogenous stand of naturally occuring *Q. fusiformis* interspersed with *Q. texana, Q. marilandica* (blackjack oak), and *Ulmus crassifolia* (cedar elm). The soils representative of this area are shallow, loamy-clays featured by hilly limestone outcrops.

Fungal mat formation. Fungal mats with spores are found under the bark of wilted Spanish oaks in Texas; no mat has been found on live oaks (1). In September 1983 and 1984, 11 and 14 diseased Spanish oaks, respectively, were located for monthly inspections. Two blackjack oaks also were observed in 1984. The trees chosen in 1983 were all in advanced stages of wilting, with 95-100% of each crown exhibiting oak wilt symptoms. However, in none of the selected trees was the cambium of major limbs and trunks necrotic, nor was the sapwood in an advanced stage of drying. Previous observations have demonstrated that such trees are unsuitable for mat formation (5). In 1984, the trees chosen were in different stages of symptom development, varying from partial wilting (5%) to complete wilting (100%). Table 1 contains the average values of symptom development for the two groups of trees.

Monthly inspections involved examining trunks for the typical hollow cavities created when the pressure cushion, in the center of the mat, forced pockets of bark from the sapwood surface. Bark was removed to confirm the presence of a mat.

Seasonal susceptibility of live oak. Thirty live oaks in each of four locations within the Burnet study site were inoculated with *C. fagacearum* on 31 May 1983; 9 September 1983; 3 January 1984; and 3 March 1984. Each location had 10 branch, 10 trunk, and 10 root-inoculated trees. In addition, four trees per infection court in each location were inoculated with sterile distilled water to serve as controls.

Inculum consisted of a spore suspension (1 x 10^6 spores/ml) prepared from 20 - 30-day-old cultures of a Texas *C. fagacearum* isolate (B206) grown in petri plates on Barnett's agar (2) at room temperature. Wounds were made with a sterile chisel and a few drops of inoculum were introduced into the exposed sapwood. Trunk inoculations were made approximately 3 m above ground. Root inoculations were made in major lateral roots approximately 0.25 - 0.5 m from the trunk. The roots were either exposed by digging or previously exposed through natural erosion. All branch inoculations were made in small branches less than 0.5 cm dia.

Inoculated trees were observed monthly for the appearance of oak wilt symptoms (1), confirming successful inoculations. The incubation periods in Table 2 represent the minimum time for symptoms to be observed on any trees within an inoculation group.

Nitidulid trapping. Flight traps were placed in two locations (A and B) at the Burnet site, approximately 300 m apart, with 12 traps on five trees in each location. The arrangement consisted of attaching four traps to a centrally located, diseased Spanish oak and two additional traps on oaks 18 m distant in cardinal directions.

The trap design was that of Skalbeck (18), consisting of a widemouth jar suspended with angle-iron from the trunk of a tree. The design allowed flying insects to enter the jar top through a 7-mm wire screen and an inverted paper funnel. Masonite squares were suspended over the jar to exclude rain. Bait was a mixture of corn syrup, water, and baker's yeast (0.5 L, 0.5 L, and 5.0 gm., respectively) contained in a small plastic bottle. Brass-wire cloth was fitted in a hole cut in the lid, allowing insects to detect the bait.

The trapping period extended from 5 April 1984 - 24 April 1985. Traps were collected and bait was replaced on a weekly basis except during November - January, when traps were replaced bi-weekly. Collected traps were returned to the laboratory where insects were sorted and

counted.

Results

Mat formation. During 1983 - 1984, fungal mats first formed in November on three of the 11 diseased Spanish oaks (Table 1). In December, fresh mats were found on five trees. In some cases, the same tree would continue to bear fresh mats for two months following the initial discovery. Although none was found in February, peak mat formation occurred in March on eight trees. April was the last month of mat formation during the 1983 - 1984 season.

Fungal mat formation followed a similar trend during 1984 - 1985, although a lesser proportion of trees in the sample had mats (Table 1). March was again the peak month, when five of the 14 trees had fresh mats. May was the final month of mat formation in 1985. The same year, fruiting structures of the canker fungus, *Hypoxylon atropunctatum,* were found on four trees, and six other trees resprouted along the trunks the following spring. No mat formed on the two diseased blackjack oaks.

Seasonal susceptibility of live oak. Of the 72 live oaks inoculated with *C. fagacearum,* 26 developed oak wilt symptoms (Table 2). The shortest incubation period was 55 days for trees inoculated in roots and trunks in May 1983. The longest incubation time was 294 days following root inoculations in September 1983. Some trees became diseased after inoculations in each of the four months, ranging from 13 (73%) following the May inoculations to 2 (11%) for those inoculated in January.

Only one branch inoculation, made in May, resulted in symptoms. No other tree became infected as a result of branch inoculation. All trunk and root inoculations in May caused infection (Table 2). A high success rate for trunk and root inoculations (83% and 50%, respectively) was achieved also in March.

Of the 48 trees inoculated with sterile distilled water, one became infected with *C. fagacearum.* This tree was root inoculated in May, and became infected probably as a result of root-root transmission from nearby, naturally-infected trees.

Nitidulid trapping. A total of 1,227 free-flying nitidulids was captured in Site A during the 13 month trapping period while 834 were trapped in Site B. The peak month of nitidulid activity for Site A was April 1984 (56 beetles/tree) and May 1984 (41 bettles/tree) for Site B (Fig. 1). Numbers of nitidulids captured decreased after May through the summer and fall. Only 7 and 4 beetles were captured during November through January at sites A and B, respectively. February was a month of increasing nitidulid activity, but the average numbers of nitidulids captured in spring of 1985 were less than the number captured in 1984.

Discussion

The results of these studies implicate nitidulid beetles and spring inocluations as partially responsible for the growing oak wilt epidemic in

Table 1. Formation of fungal mats by *Ceratocystis fagacearum* on naturally infected Spanish oaks during 1983 - 1984 and 1984 - 1985 at Burnet, TX.

Species	No. trees	Average symptom extent (%)[x]	Numbers of trees bearing fresh mats							
			Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
						1983 - 1984				
Quercus texana[y]	11	99	0	3	5	2	0	8	2	0
						1984 - 1985				
Q. texana	14	67	0	0	0	1	3	5	0	1

[x]Indicates amount of crown affected by symptoms in September.
[y]Spanish oak.

Texas. Long distance transmission of *C. fagacearum* by insect vectors is extremely important because it initiates new centers of infection. In terms of numbers of trees killed, root graft transmission and spread through common root systems may be more important (1, 9). Also, there may be additional, undetected insect vectors involved in spread of the pathogen that do not rely on mat formation or natural wounding for successful transmission.

February through May appears to be the period of greatest risk for *C. fagacearum* transmission in Texas. During both years of this study, inoculum availability for acquisition of spores by insect vectors was greatest in March. Mat formation coincided with increases in activity of sap-feeding nitidulid beetles recorded in both years. March and May were the months of maximum susceptibility of live oaks to wound inoculation of trunks and roots. Therefore, pruning and other kinds of wound-inducing practices should be avoided during February-May to prevent long-distance fungus transmission. If wounding is unavoidable, then the prompt application of dressings is justified to prevent nitidulids from introducing spores into wounds on healthy trees. The weathered wound paints do not need to be reapplied because only fresh wounds, up to 4-5 days old, are receptive to infection (9). Also, the type of wound dressing is unimportant, assuming they are not phytotoxic and beetles are denied access to wounds (11).

The oak wilt disease cycle in Texas resembles the syndrome as it occurs in other states. For example, the seasonal pattern of fungal mat formation on diseased Spanish oaks is consistent with

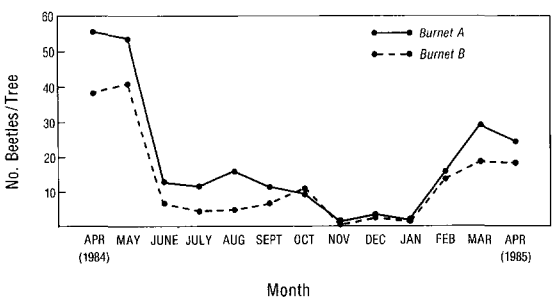

Figure 1. Average values of nitidulid populations trapped per tree at two sites (A and B) in the Burnet study area. There were five trees and 12 traps in each site.

previous observations. The extent of symptoms averaged 99% for the 11 oaks chosen for examination in September 1983 (Table 1). In September 1984, the 14 selected Spanish oaks had a lower value of 67% for the average extent of crown symptoms. Only 35% of the trees developed fungal mats in 1985, whereas 73% developed mats in 1984. Many of the trees chosen were not suitable for mat development in 1985. Mat formation observed in other states has proven highly variable, with reports ranging from 5% on diseased oaks in Missouri to 80% on trees in southern West Virginia (9, 17). Moderate temperatures and high sapwood moisture content are conducive for mat formation (5). It is unlikely, then, that mats will be found forming after May in central Texas because daily temperatures begin to rise and rainfall becomes sporadic. An additional factor limiting mat development was colonization by the canker fungus *H. atropunctatum.* Competing organisms can prevent saprophytic survival of *C. fagacearum* in the sapwood of dead and dying trees (20), thus preventing the formation of mats by the fungus. *Hypoxylon atropunctatum* limits the spread of *C. fagacearum* in Arkansas (19).

The incubation periods were as short as 55 days for May inoculations and extended up to 294 days for root inoculations made in September.

Table 2. Rates of infection on live oaks (*Q. fusiformis*) artifically inoculated with *Ceratocystis fagacearum* in the branches, trunks and roots during four months.

		No. trees with symptoms/No. inoculated		
Inoculation date	*Infection court*	*Ceratocystis fagacearum*	*Controls (distilled water)*	*Incubation times (days)*
	Branch	1/6	0/4	67
31 May 83	Trunk	6/6	0/4	55
	Root	6/6	1/4	55
	Branch	0/6	0/4	--
9 Sept. 83	Trunk	1/6	0/4	229
	Root	2/6	0/4	294
	Branch	0/6	0/4	--
3 Jan. 84	Trunk	1/6	0/4	203
	Root	1/6	0/4	203
	Branch	0/6	0/4	--
15 Mar. 84	Trunk	5/6	0/4	131
	Root	3/6	0/4	131

These incubation periods are also consistent with results of inoculation experiments obtained from other studies. In tests examining inoculum load and symptom development in artificially inoculated black oaks (*Q. velutina*), incubation times ranging from 14 to 308 days were observed (10). Bole inoculations caused higher infection rates than branch inoculations, just as they did in the present study. In general, oaks are considered most susceptible to natural infection by *C. fagacearum* during periods of springwood vessel development and leaf expansion, although artificial inoculations can be successful during most times of the year (9).

Due to the dependence of nitidulids on mat formation for inoculum acquisition, and wounding for introducing the pathogen, the long distance transmission of *C. fagacearum* in central Texas is probably a relatively rare event. Therefore, spraying insecticides to prevent nitidulids from visiting healthy trees would not be practical for oak wilt control. Nitidulid transmission can be prevented by destroying diseased Spanish oaks to eliminate sources of inoculum. This measure, when combined with reduced wounding of oaks in the spring and application of wound dressing, should be sufficient to decrease the rate of spread of the oak wilt epidemic in a given area. Caution should also be taken to avoid removing firewood from diseased stands. These recommendations are most appropriate in the Edwards Plateau region of central Texas, west of the Balcones escarpment where diseased Spanish oaks are commonly found and where the present studies were conducted.

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Department of Plant Pathology and Microbiology
Texas A&M University
College Station, TX 77843-2132