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Nitidulids as vectors of the oak wilt fungus and other Ceratocystis spp. in Texas

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Abstract

Sap-feeding nitidulid beetles were trapped for 15 months at four oak wilt centers in central Texas and assayed for the presence of *Ceratocystis fagacearum*. Levels of contamination with the pathogen ranged 0.3 % (Kerrville) – 2.0 % (Austin); also detected were *C. piceae* at slightly higher levels and a low number of beetles carrying *C. pluriannulata*. The percentages of contaminated beetles were similar to those reported for other parts of the oak wilt range in the USA, but probable species differences in Texas were detected.

1 Introduction

Sap-feeding nitidulids (Order: Coleoptera, Family: Nitidulidae) have been shown to be vectors of the oak wilt fungus (*Ceratocystis fagacearum* Bretz Hunt) (Dorsey et al. 1951; Jewell 1956; Norris 1953; Himelick et al. 1958). Numerous ecological studies involving tree wounding, nitidulid contamination and behavior, and inoculum availability have fulfilled the guidelines for verification of a vector relationship (Leach 1940).

The only known inoculum source responsible for contamination of nitidulids with *C. fagacearum* is a mycelial mat that forms beneath the bark of diseased red oaks (Erythrobalanus). The fungal mats produce abundant conidia and sometimes perithecia with ascospores. The limited production of mats in the southern and western range of oak wilt has implied the existence of other vectors (Rexrode 1976). There are also convincing reports that bark beetles from the genus *Pseudopityophthorous* can acquire and transmit propagules of *C. fagacearum* directly from the sapwood of infected trees to feeding wounds on healthy oaks (Berry and Bretz 1966; Rexrode and Jones 1970). These *C. fagacearum*-insect vector systems are considered to be inefficient due to limited exposure of insects to inoculum and unreliable infection courts (MacDonald and Hindal 1981; Rexrode and Jones 1970).

Recently, destructive outbreaks of oak wilt in a 36 county area of central Texas have been observed and documented (Appel and Maggio 1984; Appel et al. 1987). Prior to these reports, our understanding of oak wilt was derived from the disease as it occurs in deciduous oaks. Live oaks (*Quercus fusiformis* Small and *Q. virginiana* Mill.) comprise a majority of the native oak population in central Texas and grow under high temperatures previously considered to limit the growth and survival of *C. fagacearum* (MacDonald and Hindal 1981). The high incidence of the disease in Texas is atypical when compared to most other states, especially since mycelial mats fail to form on live oaks. Mats do form on two native red oaks in Texas, Spanish oak (*Q. texana* Buckl.) and blackjack oak (*Q. marilandica* Muenchh.), but these comprise only a small proportion of the Texas oak population.

The introduction of *C. fagacearum* into new areas may result in establishing more efficient vector relationships than the ones already known (Gibbs 1981; Ruetze and Parami-

WARAN 1984). Insect transmission in the epidemic of oak wilt in the live oak savannahs of central Texas has not been thoroughly investigated. The following describes the vector relationships between nitidulid beetles, *C. fagacearum*, and other *Ceratocystis* spp. found during a previous survey of nitidulids in central Texas (APPEL et al. 1986).

2 Materials and methods

A modified trap design (SKALBECK 1976) was used to capture free-flying nitidulids at four locations in central Texas (Fig. 1). Traps at all locations were positioned 1.4 m above ground. The greatest number of traps was located in a large oak wilt center near Burnet, TX. This disease center appeared to be a conglomerate of infection foci covering ca. 20 ha and consisted of a mixed stand of live oaks (*Q. fusiformis* and *Q. virginiana*), Spanish oak (*Q. texana*) and blackjack oak (*Q. marilandica*). On three different dates (Table 1), traps were established on 5 Spanish oaks, 19 live oaks and one fence post. The second site was located in a mixed oak urban environment in Austin, TX. The traps were placed on three live oaks in a small disease center. In the third site, located in Kerrville, TX, the traps were placed on two Spanish oaks and one live oak. The final trapping site was on the perimeter of a 15 ha disease center consisting of only live oaks at Round Rock, TX. This site was chosen due to the lack of red oaks, thereby eliminating the possibility of mycelial mats as the local source of inocula.

On sampling dates, the traps containing insects were removed and stored in ice chests until the beetles could be refrigerated in the laboratory. Insects were then identified to species, put in gelatin capsules and frozen until processing.

A spermitization assay was used to detect the presence of viable *C. fagacearum* spores (STAMBAUGH, FERGUS and CRAIGHEAD 1955) by plating suspensions of crushed beetles onto cultures of both sexual mating types. The *C. fagacearum* isolates were grown for 10 days on a modified version of Barnett's agar medium (BARNETT 1953). This medium substituted casein hydrolysate for the casamino acids, used half the amount of potassium phosphate (0.5 gm) and 1.0 gm each of yeast extract and thiamine HCL per liter volume. Each beetle was crushed and ground with a flamed rod in a test tube containing 1 ml of sterile distilled water. Half the volume (0.5 ml) of the suspension was spread on two cultures each of both the A and B sexual mating types of *C. fagacearum*. A nitidulid was considered contaminated with the pathogen when fertile perithecia were observed. The assay cultures were periodically tested for fertility by crossing with the opposite sexual mating type. Other *Ceratocystis* species were identified by the formation of characteristic fruiting structures (CRANE and SCHOKNECHT 1973; UPADHYAY 1981) on the spermitization

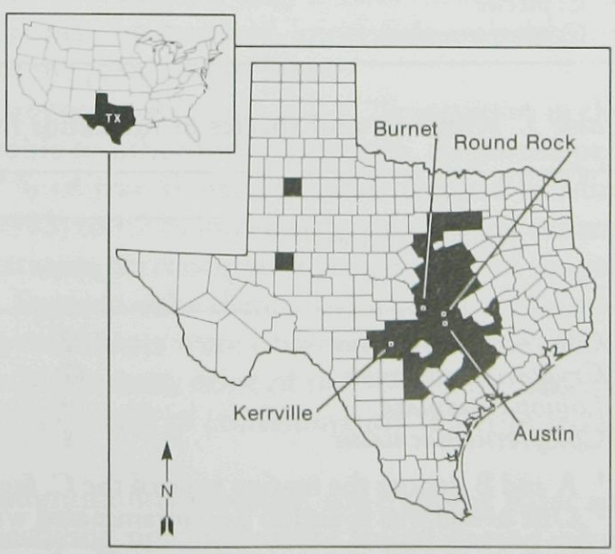

Fig. 1. Map of Texas with range of oak wilt (shaded counties) and the four nitidulid trapping locations

Table 1. **Locations and sampling design for collection of nitidulid beetles in Texas oak wilt centers**

Location	No. trees	No. traps	No. collections	Collection dates
Round Rock	6	12	26	Oct. 4, 1984 – May 10, 1985
Austin	3	12	49	Apr. 19, 1984 – May 10, 1985
Kerrville	3	12	20	Apr. 12, 1984 – May 10, 1985
Burnet A	4	16	54	Mar. 9, 1984 – May 10, 1985
Burnet B	11	20	51	Apr. 5, 1984 – May 10, 1985
Burnet C	9	18	26	Oct. 4, 1984 – May 10, 1985

plates in addition to growth characteristics and sporulation on the following culture media; acidified potato dextrose agar, cornmeal steep agar (100.0 gm stone-ground cornmeal/L distilled H_2O amended with 2 gm yeast extract), modified malt extract agar (Upadhyay 1981) and pablum agar (Olchowecki and Reid 1973).

3 Results

A total of 4126 beetles, representing 85% of the total 4821 trapped, were assayed for *Ceratocystis* contamination; 0.65% tested positive for *C. fagacearum*. The majority of beetles assayed (3293) were from the Burnet site (Table 2). Twenty-three of the beetles, or 0.6%, were contaminated with *C. fagacearum* at the Burnet site. A higher amount, 4.2%, were contaminated with *C. piceae* (Munch) Bakshi, and a lesser proportion (0.3%) were contaminated with *C. pluriannulata* Hedgc. at Burnet. *Ceratocystis piceae* was easily identified by the prolific synnemata production of its anamorph, *Pesotum piceae* Crane and Schoknecht and its mononematous *Sporothrix* state with numerous ramoconidia (Crane and Schoknecht 1973). *Ceratocystis pluriannulata* was identified by its distinct perithecial characters (Upadhyay 1981).

Nitidulids contaminated with *C. fagacearum* were also trapped at Kerrville and Austin, but none was found at Round Rock. The highest level of pathogen contamination was in

Table 2. **The percentages of nitidulids from four sites in central Texas found to be contaminated with Ceratocystis fagacearum, C. piceae, and C. pluriannulata**

	Percentages (%) of contaminated nitidulids			
	Kerrville	Austin	Burnet	Round Rock
C. fagacearum	0.3	2.0	0.6	0.0
C. piceae	2.5	2.8	4.2	4.6
C. pluriannulata	0.0	1.4	0.3	1.9

Table 3. **Numbers and species of nitidulids from which Ceratocystis species were revovered**

Nitidulid species	Number of beetles			
	C. piceae	*C. pluriannulata*	*C. fagacearum*[1]	
			A	B
Colopterus maculatus	102	4	3	6
Cryptarcha concinna	47	7	4	14[2]
Lobiopa undulata	9	0	1	1[2]
Colopterus maculatus	8	5	0	0

[1] A and B denote the mating type of the *C. fagacearum* isolate recovered.
[2] One beetle was found to be contaminated with both mating types.

Austin, where 2% of the 146 beetles tested positive. More nitidulids were contaminated with *C. piceae* than with other *Ceratocystis* species at each site.

Three nitidulid species were contaminated with the oak wilt pathogen, including 18 *Cryptarcha concinna* Melsh., 9 *Colopterus maculatus* Erich., and 2 *Lobiopa undulata* Say (Table 3). Although both mating types of the fungus were found, a majority were of type B (Table 3). Two of the collected beetles carried spores of each mating type. The same three nitidulid species plus *Colopterus truncatus* Rand. were contaminated with *C. piceae*. The majority of the *Ceratocystis piceae*-contaminated beetles were *C. maculatus* and *C. concinna*. Contamination with *Ceratocystis pluriannulata* was relatively low, but only on *L. undulata* was this fungus never detected.

The highest levels of nitidulid contamination for all three *Ceratocystis* species were observed in April of each year (Fig. 2). *Ceratocystis fagacearum* was found on beetles assayed from the first five months of trapping (March–July, 1984), but was not found from August 1984 (Month 6) through March 1985 (Month 13). *Ceratocystis piceae* was found on beetles trapped during every month, while contamination with *C. pluriannulata* only occurred on beetles trapped from March through May of 1985.

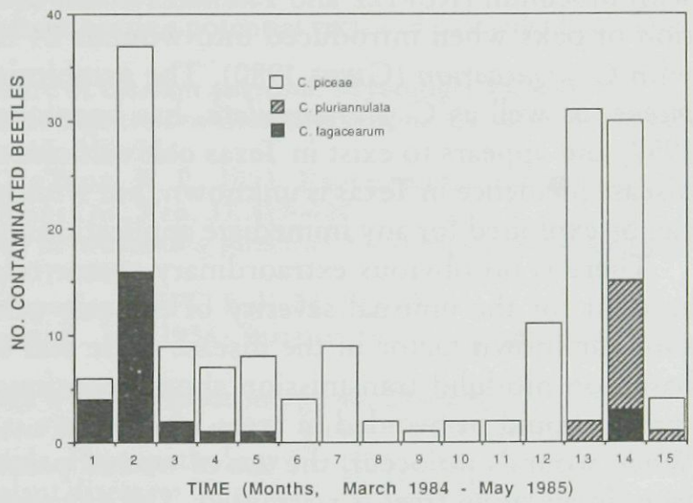

Fig. 2. Numbers of trapped beetles found contaminated with *Ceratocystis piceae, C. pluriannulata*, or *C. fagacearum* during March 1, 1984 through May 15, 1985

4 Discussion

The local transmission of *C. fagacearum* through functional root connections is probably responsible for most oak wilt losses of live oak in Texas (Appel 1986). The initiation of new foci by long-distance vector transmission appears to be a relatively rare event, but is nonetheless an extremely important consideration in the epidemiology and control of the disease. An oak wilt management program will be successful only if both means of transmission are addressed. Although other vectors may be involved, nitidulids are probably the principle overland, long distance vectors of the pathogen in Texas.

In many respects, the vector relationship between nitidulids and *C. fagacearum* in the live oak savannahs of central Texas is comparable to other forest ecotypes. Reported percentages of contamination ranged from 0.67% of free-flying nitidulids trapped in Minnesota oak wilt centers (Juzwik and French 1983) to 1.7% in Pennsylvania disease centers (Yount et al. 1955). At three of the Texas locations, percentages of contamination ranged between 0.3% (Kerrville) to 2.0% (Austin). Spanish oaks comprised a small portion of the diseased population at these sites, and mycelial mats were observed in the immediate vicinities of the traps at Kerrville and Burnet. In contrast, none of the 364 beetles assayed from the Round Rock site were contaminated. This lack of contamination is attributed to the lack of red oaks and mat formation.

In addition to the numbers of contaminated nitidulids detected, the trapping dates are also similar to previous reports. Althoug free-flying nitidulids were found during other

seasons (Appel et al. 1986), all of the nitidulids carrying *C. fagacearum* in Texas were trapped during the spring and early summer. These months coincide with periods of maximum fungal mat formation in Texas (Appel et al. 1987).

The species of nitidulids responsible for *C. fagacearum* transmission in Texas appear to be different than those identified elsewhere. According to the numbers of beetles trapped and found to be contaminated, *Colopterus maculatus* and *Cryptarcha concinna* were the most likely vectors of the pathogen at the study sites (Table 2). These species have been found in oak wilt centers elsewhere, but *Glischrochilus* spp., *Epuraea* spp. and *Colopterus truncatus* Rand are usually the nitidulids implicated as vectors (Himelick et al. 1954; Jewell 1956; Juzwik and French 1983). In addition to long distance transmission, nitidulids in Texas may be important to the sexual reproduction of *C. fagacearum*. In two instances, both mating types were found on one beetle, indicating the interaction between beetles and opposite mating types of the pathogen.

Ceratocystis piceae may operate as a biological control agent for oak wilt in two stages of the life cycle of *C. fagacearum*. Mycelial mats may become overgrown by *C. piceae* within a few days following bark cracking, thus limiting the contact nitidulids can make with inoculum (Ruetze and Parameswaran 1984). Also, *C. piceae* has prevented infection of oaks when introduced into wounds 24 h before inoculation of the same wound with *C. fagacearum* (Gibbs 1980). The symbiotic relationship between nitidulids and *C. piceae*, as well as *C. pluriannulata*, has been observed previously (Jewell 1956; Shigo 1958) and appears to exist in Texas oak wilt centers. The impact of these relationships on disease incidence in Texas is unknown, but without further research it is unlikely that they can be exploited for any immediate applications in disease control.

There is no obvious extraordinary vector relationship operating in Texas that might account for the unusual severity of the oak wilt epidemic. Although the possibility of some unknown factor in the disease cycle still exists, certain control recommendations based on nitidulid transmission should continue to be made. Wounding of oaks in the spring should be avoided in areas where there is a potential for mycelial mat formation. When wounds do occur, the use of wound paints to discourage contaminated nitidulids from inoculating trees is warranted. The movement of wood from diseased stands should also be discouraged to prevent the transport of fungal mats or contaminated nitidulids to new locations.

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Summary

Sap-feeding nitidulid beetles were trapped in four oak wilt centers in central Texas and assayed for contamination with the pathogen, *Ceratocystis fagacearum*. No contaminated beetle was found at one location, while levels of contamination ranged 0.3 % to 2.0 % at the remaining three. All of the beetles carrying the fungus were found in spring and early summer. Three nitidulid species were found to be contaminated. In addition to *C. fagacearum*, beetles were also found to be carrying *C. piceae* and *C. pluriannulata*.

Résumé

Les Nitudilidés comme vecteurs de l'agent de l'Oak wilt et d'autres Ceratocystis spp. au Texas

Les insectes nitudilidés consommateurs de sève ont été capturés dans quatre foyers d'Oak wilt au Centre du Texas, et analysés pour leur contamination par *Ceratocystis fagacearum*. Dans un site, aucun insecte porteur n'a été trouvé, alors que le taux d'insectes porteurs allait de 0,3 % à 2,0 % dans les autres sites. Les insectes porteurs n'ont été trouvés qu'au printemps et début d'été. Trois espèces de nitudilidés ont été trouvées contaminées. Outre le *C. fagacearum*, les insectes étaient également porteurs de *C. piceae* et *C. pluriannulata*.

Zusammenfassung

Nitiduliden als Überträger von Ceratocystis fagacearum und anderen Ceratocystis-Arten in Texas

In vier Zentren der Eichenwelke in Mitteltexas wurden Käfer aus der Familie der Nitidulidae in Fallen gefangen und auf Sporen von *Ceratocystis fagacearum* hin untersucht. An einem Ort wurden keine Inokulum tragenden Käfer gefunden, an den drei übrigen Orten lag die Kontaminationsrate zwischen 0,2 und 2 %. Käfer, die Inokulum des Pilzes trugen, traten nur im Frühjahr und Frühsommer auf. Drei Nitiduliden-Arten wurden als Träger von Pilzinokulum nachgewiesen. Außer *C. fagacearum* wiesen die Käfer Inokulum der Pilze *C. piceae* und *C. pluriannulata* auf.

References

Appel, D. N., 1986: Recognition of oak wilt in live oak. J. Arboric. **12**, 213–218.

Appel, D. N.; Maggio, R. C., 1984: Aerial survey for oak wilt incidence at three locations in central Texas. Plant Disease **68**, 661–664.

Appel, D. N.; Andersen, K.; Lewis, R., 1986: Occurrence of nitidulid beetles (Coleoptera: nitidulidae) in Texas oak wilt centers. J. Econ. Entomol. **79**, 1276–1279.

Appel, D. N.; Peters, R.; Lewis, R., 1987: Tree susceptibility, inoculum availability, and potential vectors in a Texas oak wilt center. J. Arboric. **13**, 169–173.

Berry, F. H.; Bretz, T. W., 1966: Small oak bark beetle a potential vector of oak wilt. Plant Dis. Rep. **50**, 45–49.

Barnett, H. L., 1953: A unisexual male culture of *Chalara quercina*. Mycologia **45**, 450–457.

Crane, J. L.; Schoknecht, J. D., 1973: Conidiogenesis in *Ceratocystis ulmi, Ceratocystis piceae*, and *Graphium penicilloides*. Amer. J. Bot. **60**, 346–354.

Dorsey, C. K.; Jewell, F. F.; Leach, J. G.; True, R. P., 1953: Experimental transmission of oak wilt by four species of Nitidulidae. Plant Dis. Rep. **37**, 419–420.

Gibbs, J. N., 1980: Role of *Ceratocystis piceae* in preventing infection by *Ceratocystis fagacearum* in Minnesota. Trans. Br. Mycol. Soc. **74**, 171–174.

– 1981: European Forestry and *Ceratocystis* species. EPPO Bull. **11**, 193–197.

Himelick, E. B.; Curl, E. A.; Zuckerman, B. M., 1954: Tests on insect transmission in Illinois. Plant Dis. Rep. **38**, 588–590.

Jewell, F. F., 1956: Insect transmission of oak wilt. Phytopathology **46**, 244–257.

Juzwik, J.; French, D. W., 1983: *Ceratocystis fagacearum* and *C. piceae* on the surfaces of free-flying and fungus-mat-inhabiting nitidulids. Phytopathology **73**, 1164–1168.

Leach, J. G., 1940: Insect transmission of plant diseases. New York: McGraw-Hill. 615 pp.

MacDonald, W. L.; Hindal, D. F., 1981: Life cycle and epidemiology of *Ceratocystis*. In: Fungal Wilt Diseases of Plants. Ed. by M. E. Mace and A. A. Bell. New York: Academic Press. pp. 113–144.

Norris, D. M., 1953: Insect transmission of oak wilt in Iowa. Plant Dis. Rep. **37**, 417–418.

Olchowecki, A.; Reid, J., 1973: Taxonomy of the genus *Ceratocystis* in Manitoba. Can. J. Bot. **52**, 1675–1711.

Rexrode, C. O., 1976: Insect transmission of oak wilt. J. Arboric. **2**, 61–66.

Rexrode, C. O.; Jones, T. W., 1970: Oak bark beetles – important vectors of oak wilt. J. For. **68**, 294–297.

Ruetze, M.; Parameswaran, N., 1984: Observations on the colonization of oak wilt mats (*Ceratocystis fagacearum*) by *Pesotum piceae*. Eur. J. For. Path. **14**, 326–333.

Shigo, A. L., 1958: Fungi isolated from oak wilt trees and their effects on *Ceratocystis fagacearum*. Mycologia **50**, 757–769.

Skalbeck, T. C., 1976: The distribution of Nitidulidae in deciduous forests of Minnesota. Ph. D. Dissertation, Univ. Minnesota, St. Paul, MN. 257 pp.

Stambaugh, W. J.; Fergus, F. C.; Craighead, F. C., 1955: Viable spores of *Endoconidiophora fagacearum* from bard and wood-boring beetles. Plant Dis. Rep. **39**, 867–871.

Upadhyay, H. P., 1981: A monograph of *Ceratocystis* and *Ceratocystiopsis*. Athens: The University of Georgia Press. 176 pp.

Yount, W. L.; Jeffery, A. R.; Thompson, H. E., 1955: Spores of *Endoconidiophora fagacearum* on the external surface of the body of nitidulids. Plant Dis. Rep. **39**, 54–57.

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