5. Trunk Injury and Fungal Transport by *Agrilus bilineatus*, *Chrysobothris femorata*, and *Xyloterinus* Sp. in Oak Wilt-Infected Trees in Texas.

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Abstract. Examinations for trunk borers revealed that over 95% of the oak wilt (*Ceratocystis fagacearum*)-infected trees in Texas were infested with twolined chestnut borers (*Agrilus bilineatus*) and flatheaded appletree borers (*Chrysobothris femorata*). Infestations by the ambrosia beetle (*Xyloterinus* sp.) were also common. The amount of borer injury varied, but in some trees it was sufficient to cause additional stress to trees already weakened by oak wilt. Variations in the amount of xylem injury by these two borers might help explain why some trees die more rapidly than others following oak wilt infections. In Texas, adult twolined chestnut borers were emerging during the first week of March, and a peak number was collected on sticky traps in oak wilt infection centers in June. Isolations from 81 adult ambrosia beetles, 40 twolined chestnut borers (22 larvae and 18 adults), and 17 flatheaded appletree borers (larvae) did not yield *C. fagacearum*, even though the fungus was isolated from the wood from which these insects were collected. These insects are not likely vectors of *C. fagacearum* because they carry faster growing fungi and attack trees already stressed by oak wilt or other diseases.

Additional key words: Ambrosia beetles, *Ceratocystis fagacearum*, flatheaded appletree borer, and twolined chestnut borer.

A study was initiated in 1978 to identify the role of insects in the decline and mortality of oak trees (*Quercus* spp.) in Texas. However, *Ceratocystis fagacearum* (the oak wilt fungus) rather than insects was identified as the primary cause of the decline and mortality (5). Trees with oak wilt either died within a few weeks after infection or slowly died back over a period of several years (5). The reason for this variation in tree response to oak wilt has not been explained. The premise used for further study was that trunk-wounding insects may cause additional injury to some of the oak-wilt infected trees and hasten tree mortality. Also, it was postulated that some of the trunk wounding insects may actually transmit the oak-wilt fungus. This report is about the amount of injury caused by trunk-boring insects in Texas and their potential for transmitting *C. fagacearum*.

Materials and Methods. Strips of screen wire (15 X 30 cm) coated with

sticky trap material (Tree Tanglefoot^{TM})[1] were hung on oak tree trunks to collect active trunk boring insects in oak wilt infection centers at Kerrville, Texas. Two traps were hung on each of 15 Texas live oak (*Quercus fusiformis*) trees in April 1978. Monthly collections were made through July and the number of identifiable adults recorded.

Bark (about 450 cm^2) was removed from branches and boles of about 100 oak wilt-infected trees to detect injury by trunk boring insects in 1978-83. Trunk borers and the type of tissue injured by them were identified. In 1984, 49 dead (due to oak wilt), 19 wilting, and 4 healthy trees were examined for tree borer injury. Only four healthy trees were used because tree wounding and bark removal expose high value trees to sap feeding beetles that might transmit *C. fagacearum*. In addition, a 450-cm^2 bark section was removed from the lower 4 m of each tree trunk to expose borer injury. Texas live oaks, Texas red oaks (*Q. shumardii* var. *texana*), and black-jack oaks (*Q. marilandica*) were examined.

Isolations were made from living borers taken from bole sections of oak wilt-infected Texas live oaks to detect contamination by *C. fagacearum*. Borer larvae were removed from underneath the bark of bole sections after they were transported to the laboratory in an ice chest. The larvae were surface disinfected by submerging them in 1.05% (a.i.) sodium hypochlorite for 30-90 seconds. Afterwards, they were aseptically sectioned (cross sections) into eight parts each and transferred to potato dextrose agar (PDA) in petri dishes. Isolations were made from 176 sections of 22 twolined chestnut borers (*Agrilus bilineatus*) and 136 sections of 17 flatheaded appletree borers (*Chrysobothris femorata*). Four sections were placed in each petri dish. Isolations were also made from bolewood of five trees infested with these borers, including small strips of wood engraved with twolined chestnut borer galleries, and fecal pellets with frass. Bolewood chips were treated with sodium hypochlorite before placing them on PDA, but fecal pellets with frass were not treated. Fungal identifications were made after 2 weeks.

Isolations were made from adult borers when they emerged from trunk sections of oak wilt-infected wood. Insects were collected in April 1981 when they emerged from tree trunk sections of a Texas live oak that had wilted in autumn of 1980. The wood sections were encaged with small mesh screen, placed in a growth chamber, and subjected to alternating periods of 12 hours of light at 26°C and 12 hours of dark at 16°C. Each insect was placed in a sterile petri dish and refrigerated until used for isolations. Other collections were made at room temperature when adults emerged from bole sections of a wilting Texas live oak in June 1980 and a wilting Texas red oak in March 1984. Each insect was placed in a petri dish (one insect per dish) containing PDA for 30 minutes before surface disinfection with sodium hypochlorite and transferral to another petri dish containing PDA. Fungal identifications were made after 2 weeks.

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[1]The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

Results. Twolined chestnut borers (adults) were the only identifiable trunk borers collected on sticky screen traps in oak wilt infection centers at Kerrville, Texas. Most of the trapped insects were covered with sticky trap material, desiccated, or badly decomposed. Even though the traps were not placed until spring, emerging twolined chestnut borers were observed during the first week of March in central Texas. The largest number was collected in June and the smallest in July (Table 1).

Table 1. Monthly collections of twolined chestnut borers on 30 sticky traps hung on 15 live oaks in oak wilt-infection centers at Kerrville, Texas, 1978.

Month	No. beetles per month[a]	Pct of total[b]
April	5.2	9
May	19	32
June	33	55
July	2.4	4

[a]The number of beetles per month was prorated for April and July to yield the equivalent of a full month's collection because traps were hung for only a fraction of these 2 months.

[b]The precent of total is based on the total number of beetles collected in April-July.

Twolined chestnut borer larvae were observed in all of the oak wilt-infected trees that were examined in 1978-83. Also, flatheaded appletree borers were observed in the same trees. The level of infestation and amount of injury varied among trees. Borers had disrupted cambium, phloem, and outer xylem as they tunneled beneath the bark of tree branches and trunks (fig. 1). Some of the twolined chestnut borers had tunneled from lower branches to 0.7 m down into lateral roots. Twolined chestnut borers were active in trees from the time of initial development of oak wilt through the time of tree mortality. During the summer, the amount of borer injury increased with disease progression. In 1984, nearly all dead and wilting Texas live oak, Texas red oak, and blackjack oak trees exhibited trunk injury caused by twolined chestnut borers and flatheaded apple tree borers when a small patch of bark was removed from each tree trunk (Table 2). Though borer damage was not found in the four healthy trees included in the 1984 survey, twolined chestnut borers were observed in three Texas live oak trees with dieback but with no oak wilt symptoms in August 1984. No pathogenic fungi were isolated from the trees. Red oak borers (*Enaphalodes rufulus*) and carpenterworms (Prionoxystus robiniae) were commonly observed in both healthy and oak wilt-infected oak trees. Also, ambrosia beetle (*Xyloterinus* sp.) infestations were common in wilting trees. Several types of trunk-wounding insects were observed in oak wilt-infected trees, but twolined chestnut borers and flatheaded appletree borers caused the greatest amount of injury.

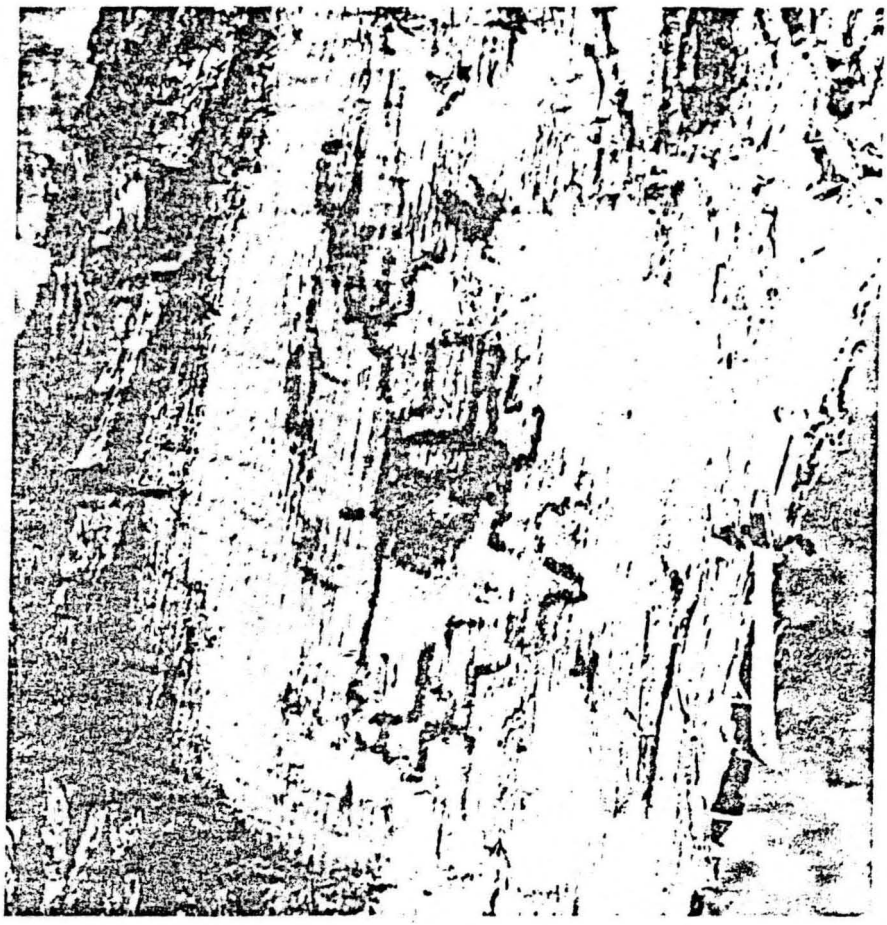

Figure 1. Texas live oak (*Quercus fusiformis*) with twolined chestnut borer (*Agrilus bilineatus*) injury beneath bark.

Table 2. Frequency of twolined chestnut borer and flatheaded appletree borer injury in oak wilt-infected oak trees in Kerrville, Texas, 1978-83.

Tree condition	No. trees	Pct of trees injured[a]
Dead due to oak wilt	49	96
Wilting	19	100
Healthy	4	0

[a]Sampled trees were randomly selected, and injury was revealed by removal of about 450 cm^2 of bark on the lower trunk. Injury by both borers was observed in affected trees.

Ceratocystis fagacearum was not isolated from the 176 twolined chestnut borer sections or the 136 flatheaded appletree borer sections. Instead, *Verticillium* sp., *Fusarium* sp., an unidentified non-sporulating fungus, and a bacterium were isolated from borers. *Ceratocystis fagacearum* was isolated from borer infested bolewood and from small pieces of wood engraved with twolined chestnut borer galleries. *Acremonium* sp. (= *Cephalosporium* sp.), *Dothiorella* sp., and *Trichothecium roseum* were isolated from fecal pellets with frass, but *C. fagacearum* was not isolated.

In April 1981, 18 adult twolined chestnut borers emerged from three live oak bole sections in a growth chamber, but *C. fagacearum* was not isolated from any of them, either before or after the surface disinfection. A total of 81 ambrosia beetles emerged from bole sections of Texas live oak and Texas red oak in June 1980 and March 1984. *Ceratocystis piceae, Trichothecium roseum,* bacteria, and several unidentified fungi were isolated, but *C. fagacearum* was not isolated from any of the insects.

Discussion. Twolined chestnut borers and flatheaded appletree borers injured the cambium, phloem, and outer xylem of trees already stressed by oak wilt infection. The amount of injury varied and might account for some of the variation in the rate of mortality for Texas live oak trees during the first 3 months after oak wilt infection.

The twolined chestnut borer is one of the most common oak infesting borers and is known to colonize wilt-infected trees in other states (7). This borer also develops in and causes injury to trees weakened by drought, insect defoliation, or frost injury (1,2,4,6,9). Flatheaded appletree borers have similar feeding habits and tree stress requirements to those of the twolined chestnut borers in oak (7). Twolined chestnut borers will not develop in trees with either desiccated or vigorously growing cambial regions (1). Therefore, trees must be alive but stressed before these borers are able to develop and cause injury. In Texas, oak wilt causes the necessary stress conditions to allow extensive borer development. As borers increase in number and grow larger, they cause more injury to vital host tissues and probably contribute to tree mortality.

Failure to isolate *C. fagacearum* from twolined chestnut borers, flatheaded appletree borers, and ambrosia beetles does not necessarily mean that these insects were free of the fungus. Stambaugh et al. (1955) reported that a small number of twolined chestnut borers and ambrosia beetles tested positive for *C. fagacearum* when the spermatization technique was used, even when the insects were collected from trees that had wilted nearly 1 year earlier (8). The spermatization technique was not used for Texas insects. It is difficult to isolate *C. fagacearum*, a slow growing fungus, from insects carrying competing and faster growing fungi.

Trunk-boring insects will not transmit the oak wilt fungus unless they deposit a sufficient quantity of relatively clean inoculum on living xylem or cambial tissues when the environment is suitable for *C. fagacearum* growth. Flatheaded appletree borers taken from pure cultures of *C. fagacearum* in petri dishes transmitted the fungus when caged on potted trees in a greenhouse. (3). However, these ideal conditions and controls are not present in the natural en-

vironment. When adult borers emerge from oak wilt-infected trees, they do not penetrate the bark of uninfected trees. Instead, they feed upon leaves and deposit eggs in bark cracks (7). When the eggs hatch and larvae penetrate into the cambial region, it is unlikely that *C. fagacearum* will be carried with them. Twolined chestnut borers, flatheaded appletree borers, and ambrosia beetles colonize and cause additional injury to trees already stressed by oak wilt, but their life habits do not accomodate transmission of *C. fagacearum*.

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