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STUDIES ON PRUNING CUTS AND WOUND DRESSINGS FOR OAK WILT CONTROL*

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ABSTRACT

Ceratocystis fagacearum causes the destructive tree disease called oak wilt. One means of pathogen spread is by insect vectors (Nitidulidae) that transmit spores into fresh wounds on healthy trees. Experiments were conducted in central Texas on native live oaks (*Quercus fusiformis*) to test pruning methods and paints on disease development. Three treatment combinations were tested on 30 trees (10 trees/treatment); flush cut unpainted, flush cut painted, and unpainted pruning cuts made according to the Shigo method. Unpainted puncture wounds were made on the lower trunks of an additional 20 trees as controls. *Ceratocystis fagacearum* spores were applied to the pruning cuts and half of the puncture wounds (positive controls) following treatment, while the other half of the punctures received distilled water as negative controls. Oak wilt symptoms first appeared in the flush cut unpainted treatment 31 days after inoculation. Infection rates, in decreasing order, were; positive control (70%), flush cut unpainted (60%), Shigo pruning method (40%), flush cut painted (20%), and negative control (10%). Pruning wounds, regardless of method, were effective infection courts for the oak wilt pathogen. Fewer trees became infected when pruning cuts were painted, but differences among infection rates for pruning cuts were not statistically significant. Tree diameters and stem aspect ratio had no bearing on infection rates. The Shigo method is recognized as a superior method for pruning, but there is no reason to change current recommendations to paint fresh wounds on susceptible oaks in high hazard oak wilt areas.

Key words: Branch protection zone *Ceratocystis fagacearum*, natural target pruning, nitidulid beetles, oak wilt, pruning paints, Shigo.

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Ceratocystis fagacearum (Bretz) Hunt, the pathogen responsible for the highly-destructive oak wilt disease, spreads in two ways (Gibbs and French 1980, MacDonald and Hindal 1981). Over

relatively short distances, spores of the fungus are drawn from diseased to healthy trees through root connections. These connections arise from grafting or from common root systems formed during vegetative propagation by root sprouts. Since root connections play an important role in oak mortality in Texas, considerable resources are expended on control in live oak (*Quercus fusiformis* Small) to prevent root transmission of the pathogen (Appel 2001, Billings, this proceedings).

The second means of spread for *C. fagacearum* is over longer distances by sap feeding beetles (Coleoptera: Nitidulidae). Inoculum sources called fungal mats form on diseased red oaks (*Quercus* subgenus *Erythrobalanus*) and provide spores for nitidulid beetles to spread to fresh wounds on healthy oaks (Norris 1953, Curl 1955, Jewell 1955, Rexrode 1976, Juzwik, French and Juzwik 1985, Appel, Peters and Lewis 1987, Appel, Kurdyla and Lewis 1990, Ambourn, Juzwik and Moon 2005). From an epidemiological perspective, the initiation of new disease centers by nitidulids is a critical stage in the oak wilt disease cycle. This means of spread is also a controversial issue for arborists throughout the range of oak wilt because pruning wounds are implicated as important infection courts for nitidulids in the oak wilt syndrome. Much of the controversy involves the recommended oak wilt control measure of applying wound dressings to prevent nitidulids from inoculating pruning cuts on susceptible trees.

Many other studies have also shown that wound dressings have some benefits when used to prevent infection from the fungal spores of various pathogens (May and Palmer 1959, Luepschen and Rohrbach 1969, Gupta and Agarwala 1972, Davis and Peterson 1973, Mercer 1979, Juzwik, French and Juzwik 1985, Biggs 1990). Luepschen and Rohrbach (1969) demonstrated that wound susceptibility of *Prunus spp.* to *Leucostoma spp.*, the pathogen causing a perennial canker disease of stone fruits, varied by time of year and that the application of shellac was beneficial in reducing infection. Similar benefits of pruning paints to control infection of *Malus spp.* with *Cylindrocarpon mali*, another canker disease of apples, have also been demonstrated (Gupta and Agarwala 1972). Not all studies, however, regarding wound dressings and their effect on disease control have been conclusive. Biggs (1990) found that wound susceptibility to infection decreases with increasing suberin and lignin formation after wounding. This varies considerably based on temperature, soil moisture, and species. After testing the effects of several post-wounding treatments, Biggs (1990) demonstrated that wound dressings, depending on type, can either hasten or retard suberin and lignin formation and infection by *Leucostoma spp.* in wounded *Prunus spp.* A number of wound dressings have been shown to inhibit the growth of *Ceratocystis fimbriata* f. *platani* (May and Palmer 1959, Davis and Peterson 1973).

In contrast to any benefits, several studies have also shown that wound dressings can be phytotoxic or non-beneficial to trees (Neely 1970, Wilson and Shigo 1973, Shigo and Shortle 1977, Shigo and Wilson 1977, Mercer 1979, Shigo and Shortle 1983, Hudler and Jensen-Tracy 2002). These studies were directed toward the use of wound dressings to prevent the ingress of decay fungi in trees. Neely (1970) showed that petrolatum, latex paint, shellac, and asphalt compounds do not promote wound closure. Shigo and Shortle (1983) tested several wound treatments in long-term experiments. They found that the treatments did not inhibit wood discoloration, and that some wound dressings could harm trees. As a result, Shigo and Shortle (1983) strongly recommended that arborists discontinue the use of wound dressings.

Due to the requirement of fresh wounds for infection by the oak wilt pathogen, wound treatments have long been a potential control measure of interest to researchers and practitioners (Drake, Kuntz and Riker 1958, Gibbs 1980). Juzwik, French and Juzwik (1985) wounded over 5,000 trees to study natural infection of oaks with *C. fagacearum*. In Minnesota, infection from

wounding occurred from May to mid-June. Numerous wounds were treated with a variety of commercially-available wound dressings including Leonard's Tree Compound (A.M. Leonard and Sons, Inc. Piqua, OH), Cabots Tree Healing Paint (Samuel Cabot Mfg., Inc., Boston, MA), and Treekote (Walter C. Clark and Son, Orange, CT). Of the 322 wounded trees treated with wound dressings, none of the trees became infected, nor did any unwounded trees contract the disease. Infection rates on untreated, wounded trees in different plots varied from 3% to 29%, depending on location and time of year the tree was wounded. As a result of these and other related studies, most educational materials developed by state and federal agencies include wound paints as part of comprehensive oak wilt control programs (Appel et al. 1995, O'Brien et al. 1999, French and Juzwik 1999, Bonello 2001, Cummings-Carlson and Martin 2005).

Wound closure has also been implicated as important to the status of oak wilt infection courts. Rates of closure have been found to be associated with how pruning cuts are made in relation to branch collars and branch attachments (Shigo 1984, 1985). In this model, branches stay separate from the parent stem from which they arise. As branches and stems increase in girth, a branch-bark ridge forms at the top of the junction of the branch and stem. Many times, there will be a swollen ring of tissue at the bottom of the branch, indicating the branch collar. Proper pruning cuts are those that involve cutting outside the branch-bark ridge (BBR) and as close to the branch collar as possible without damaging the branch collar (Shigo 1984). In addition, branches have branch-protection zones (BPZ) that limit infection in the parent stem after branch injury or removal by forming pathogen-resistant compounds within the branch tissue (Ausfess 1975, 1984, Green, Shortle and Shigo 1981, Shigo 1985).

Improper, or flush, cuts damage the tissue of the parent stem and therefore, bypass the inherent physical and chemical barriers present in the branch. Several studies have demonstrated that pruning cuts through branch collars result in increased discoloration in the parent stem outside of the branch tissue (Neely 1970, Solomon and Shigo 1976, Shigo 1984, 1985, Eisner, Gilman and Grabosky 2002). These studies have convinced many arborists to abandon the use of pruning paints when pruning oaks in areas infected with oak wilt in favor of relying on the anatomical advantages of a proper pruning cut.

An important point to note is that these studies of wound closure involved branches. Not all stem attachments comprise true branches with BPZs and branch collars. True branches, as opposed to codominant stems, can be difficult to define. Eisner, Gilman and Grabosky (2002) looked at three different criteria to determine how well branches compartmentalize discoloration associated with pruning cuts on live oaks (*Q. virginiana*). These were visible collars, pith connections between the branch and parent stems, and the aspect ratio (branch diameter to trunk diameter). Their research found that branches with visible branch collars had significantly less discolored wood after pruning. There was significantly less discoloration in pruning cuts where the piths of the branch and the parent stem did not connect. These morphological features and their influence on discoloration support findings by Shigo (1985). Most branches (89%) with visible branch collars did not have connected piths. The extent of discoloration increased as aspect ratios increased to 1 (codominant stems). In addition, branches with lower aspect ratios had fewer pith connections.

Pruning branches with aspect ratios lower than the predicted ratio (0.39) resulted in relatively small amounts of discolored wood. In a related study, Eisner et al. (2002) demonstrated that branches with lower aspect ratios, no pith connections, and visible branch collars had lower conductivity ratios, which means that these features are associated with restricted movement of water from the parent stem to the branch. They found that lower conductivity ratios result in a

decrease in discoloration, and this restriction in water flow may also reduce the infection potential of *C. fagacearum*. Studies measuring the impact of proper pruning to limit disease transmission must ensure that true branches, rather than codominant stems which do not have branch collars or BPZs, are utilized in order to accurately assess the benefits of these inherent morphological features.

To address these concerns, a study was developed to determine the accuracy of current recommendations for applying pruning paints to pruning wounds on susceptible live oaks as a precaution against vector transmission of *C. fagacearum*. The objectives of this study were 1) to determine if pruning paint served as an effective sealant to protect flush cuts from infection and 2) to determine if the physical and chemical barriers present in proper pruning cuts on branches without pruning paint were sufficient to limit infection.

MATERIALS AND METHODS

The study was conducted on an oak-woodland ranch north of Austin, TX, located at - 97°45'12"W and 30°28'23"N. The tree species on the ranch consisted mainly of live oaks (*Q. fusiformis*), but cedar elm (*Ulmus crassifolia*) and gum bumelia (*Bumelia lanuginosa*) were also present. This site was selected due to the high concentration of susceptible live oaks and the presence of oak wilt in the immediate vicinity, precluding the introduction of the disease into a new area. There were also no red oaks in the study site, so overland transmission of the fungus by vectors from other oak wilt centers was unlikely. The twelve-week study was conducted from April 30 to July 21, 2003 during a period of high susceptibility for oak wilt (Appel, Peters and Lewis 1987).

Live oaks with trunk diameters ranging from 10.0-44.5 cm (3.94-17.52 in) DBH (diameter breast height) were selected. A total of 5 treatments were implemented. Each treatment consisted of 10 trees for a total of 50 trees. The treatments consisted of: I. puncture wound - positive control, II. puncture wound - negative control, III. flush pruning cut unpainted, IV. flush pruning cut painted with pruning paint and V. proper pruning cut unpainted as described by Shigo (1984). Measurements of all branch diameters and the vertical faces of the pruning cuts were made prior to treatment. Branches used in treatment III, IV, and V had visible branch collars.

Healthy trees with stem aspect ratios (branch diameter to parent stem diameter) ranging from 0.27-0.52 were used for the pruning treatments as defined by Eisner, Gilman and Grabosky (2002). The pruning cuts were made by an International Society of Arboriculture Certified Arborist and pictures documenting each pruning cut were taken. The tree-wound dressing TreeKote Aerosol® (Walter C. Clark and Son, Orange, CT) was used to seal the 10 flush pruning cuts for treatment IV. The positive and negative control treatments (I and II) entailed wounding the tree using a disinfected screwdriver hammered into the base of the tree and pulled back to expose the vascular system. For treatments III and IV, the branch collar was cut flush to the parent limb. In treatment V, the branch collar and the branch bark ridge were not cut.

The spore suspensions used in treatments I, III, IV and V were prepared by utilizing a fresh isolate of *C. fagacearum* that was obtained from a nearby disease center in March 2003. The sample was taken from a live oak exhibiting typical oak wilt symptoms. A bole sample containing vascular xylem tissue was removed from the tree, placed on ice, and returned to the laboratory for processing. The sample chips were surface sterilized in 10% hypochlorite for 1 minute and plated onto Petri plates with potato dextrose agar (PDA) acidified with 0.1% HCL. The resulting *C. fagacearum* isolate was separated in pure culture to be used for the inoculation

treatments. A spore suspension of 2.9×10^6 conidia/ml was made on April 29, 2003 and stored in a refrigerator until inoculation.

Inoculation of treatments I, III, IV, and V were made with a few drops of the fresh spore suspension of 2.9×10^6 spores/ml on April 30, 2003. Inoculations were made early in the spring, before temperatures became too hot. High temperatures are known to limit *C. fagacearum* growth. Sterilized distilled water was used on the negative control (treatment II). The spore suspension was applied with a dropper to the basal wound for treatment I. Treatments III and V were inoculated 10 minutes after the pruning cut was made. The spore solution was brushed onto the cut surface with a sterilized paintbrush. For treatment IV, the wound was immediately sprayed with the tree wound dressing and then allowed to dry for 30 minutes. The entire pruning cut was coated with the tree wound dressing. Once the tree wound dressing was dry to touch, the wound was then inoculated with the spore suspension by using a sterile paintbrush.

Live oaks grow in groups, termed motts, consisting of highly-interconnected trees growing on common root systems and grafted roots. This growth habit complicated tree selection because the pathogen could rapidly move through the connections among treated trees and obscure the results of the treatments. The experimental design was also planned with the intention of confining the property damage to a minimal area. These conditions resulted in a limited number of available trees that had adequate aspect ratios and tree spacing, making placement of the treatments critical. In order to compensate, a buffer tree was left between the treated trees to limit movement of the fungus through root grafts into an adjacent treated tree within the same mott during the experimental period. All 50 trees were checked for symptom development every 7 days for 10 weeks.

Results from the five treatments were tested for significance by using the general linear model in SAS (SAS, Campus Drive, Cary, NC 27513) as well as the Calculation for the Chi-Square Test, an interactive calculation tool for chi-square tests of goodness of fit and independence (Preacher 2005). Each pair of treatments were tested using the chi-square calculator as well. Single factor ANOVA using MS Excel popptools (Hood 2003) was used to determine significant differences in trunk diameters, aspect ratios, areas of exposed pruning cuts, and the time of day when the inoculation was accomplished.

RESULTS

Initial symptoms in some treatments were observed 31 days after inoculation (Fig. 1). After 12 weeks, some trees became infected in each of the treatments. During the course of the experiment, typical diagnostic oak wilt symptoms (Appel et al. 1995) were regularly observed. Forty days after inoculation, veinal necrosis began to appear and tip burn of the leaves was visible. The development of symptom expression on infected trees progressed from brown leaves to tip burn to vein banding and veinal necrosis that eventually encompassed the entire crown resulting in crown loss.

At the end of the 12-week study period, significant differences were found between the positive and negative control treatments ($P=0.006$). The positive control group (treatment I) exhibited the greatest number of infected trees (70%) (Table 1, Fig. 1). The least number of infected trees after 12 weeks was in the negative controls (treatment II) where 1 tree (10%) became symptomatic. This was likely the result of the fungus spreading from a nearby infected treated tree to the negative control tree and resulted in termination of the experiment. The buffer tree between the two treatments was infected as well. The flush cut, unpainted wounds (treatment III) had the next highest infection level (60%) and were also significantly greater than the

negative control treatments ($P=0.019$). Painting the flush cuts (treatment IV) reduced the infection level to 20%, which made that treatment significantly less than the positive controls ($P=0.024$). On one of these trees that became infected, the paint was not completely dry and slipped off the wound, partially exposing the cut surface of the branch, when the spore inoculation was brushed onto the wound. This tree was immediately resprayed with pruning paint and allowed to dry before reapplication. Of the trees that were treated with the Shigo cuts (treatment V), 40% became infected as shown in Table 1. There were no significance differences among the non-flush cut treatments and the other treatments.

Based on single factor ANOVA, there were no significant differences ($P=0.6093$) among mean trunk diameters (DBH) for the 5 treatments (Table 2). When comparing the results of the flush cut painted, flush cut unpainted, and proper pruning cut treatments, there were no significant differences among stem aspect ratios (Table 3). Although more trees became infected with stem ratios of 0.3-0.39 and 0.4-0.49, there were no significant differences in percentages of infection among branch-stem ratios, ($P=0.2578$). As seen in other studies, flush cut branches resulted in larger wounds than proper pruning cuts outside the branch collar (Herring et al. 1958, Neely 1970). There were significant differences between the flush cuts that were not painted and the Shigo cuts ($P=0.10$) (Table 4). In addition, even though the maximum air temperature reached 82.5° F (Texas Commission on Environmental Quality 2003) during the day that the trees were inoculated, there were no significant differences in infection due to time of inoculation throughout the day (results not shown).

DISCUSSION

Some clear conclusions can be drawn from the results of these inoculation studies. Pruning cuts are effective infection courts for the oak wilt pathogen. Whether they are flush cuts or properly made according to the non-flush cut method, *C. fagacearum* is able to infect the wound and colonize the tree. There is some evidence that the Shigo cut may have some benefit in reducing infections, but the statistical significance is not sufficiently conclusive. Pruning paints provided greater protection, but again the differences were not statistically significant. Although neither of the measures was 100% effective in preventing infection by *C. fagacearum*, both may be useful to protect against vector-borne transmission of the oak wilt fungus. As expected, the average sizes of flush-cut pruning wounds were larger than those pruning cuts made by the Shigo method. Previous research studies illustrated that flush-cut branches resulted in larger wounds than when cuts were properly made outside the branch (Herring et al. 1958, Neely 1970). In addition to the damage to the branch collar from a flush cut, the larger wound may increase the likelihood of infection due to the greater surface area.

The results of this study need to be interpreted with an understanding of how the experimental application of spores might compare to natural conditions. Presumably, contaminated nitidulid beetles are attracted to fresh wounds by volatile compounds released from the exposed cut, just as they are attracted to certain artificial baits and pheromones (Kyhl et al. 2002). Upon arrival at the wound site, spores would be mechanically deposited on the exposed vascular system in a manner similar to wiping with a contaminated paintbrush, but at a lower concentration than that used in the artificial treatments. If wound paints are to be effective in preventing infection, they must either prevent the attractants, or volatiles, from successfully attracting the nitidulids, or they must provide a barrier to prevent the nitidulid from depositing spores, or both. As mentioned previously, the paint slipped during the inoculation of one of the trees in treatment IV, which likely resulted in the infection of that tree. If this was the case, then

the importance of using pruning paints to minimize infections related to pruning is even more critical.

Another important fact is that live oaks tend to abort their terminal buds and form codominant stems. Therefore, we found it very difficult to locate true branches within the canopies, which is why we had to use stem aspect ratios that were slightly higher than the predicted threshold ratio recommended by Eisner et al. (2002a). This observation concerning live oaks is critical. Even if proper pruning cuts were effective at limiting infection by *C. fagacearum*, pruning paints would still be required due to the high numbers of pruning cuts on stems without branch collars and BPZs. Therefore, the prudent approach for an arborist to maximize protection against infection is to make proper pruning cuts where possible and use pruning paints as an added barrier. Since vectors can theoretically infect open wounds soon after they are created, pruning paints should be applied immediately after each pruning cut.

Many of the questions left unanswered by the present study could be addressed by similar experiments with larger numbers of treated trees. Size of the pruning cut, time of year, and types of pruning paint are all important variables that should be tested. However, the opportunity to conduct inoculation experiments in Texas with the oak wilt pathogen is rare due to the potential for causing losses of large numbers of trees. Regardless of the limitations of the present study, we have no reason to warrant changing the current recommendations for oak wilt prevention in Texas. Intentional wounding (pruning) of oaks in high-risk areas for infection by *C. fagacearum* should be limited to seasons when fungal mats are not forming and the nitidulid populations are minimal. In Texas, February 1 through June 1 is considered to be an undesirable time to prune trees, but, due to climatic variation, caution should be exercised during other periods as well. Pruning paints and proper pruning are considered to be important measures to further minimize the likelihood of an infection and promote tree health.

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Table 1. Numbers of trees infected with *Ceratocystis fagacearum* per treatment type.

Treatments (No.)	Diseased ^a	Healthy	Total	P-value (Comparison)
Positive control (I)	7 a	3	10	0.006 (I, II)
Flush cut unpainted (III)	6 ab	4	10	0.019 (II, III)
Shigo cut (V)	4 abc	6	10	
Flush cut painted (IV)	2 bc	8	10	0.024 (I, IV)
Negative control (II)	1 c	9	10	

^a Numbers in column followed by the same letter are not statistically different as determined with Chi square goodness of fit at $P \leq 0.05$.

Table 2. Mean diameters at breast height (DBH) of trees within treatments ^a.

Treatments (No.)	Mean DBH (cm)	Standard Error
Positive control (I)	17.89	0.4792
Negative control (II)	17.52	0.8942
Flush cut painted (IV)	20.70	1.1187
Flush cut unpainted (III)	20.06	1.0136
Shigo cut (V)	22.26	1.0804

^a DBH among treatments were not significant at $P = 0.6093$

Table 3. Distribution of infected/treated trees by treatment and stem aspect ratios on July 21, 2003 ^a.

Treatments (No.)	Stem aspect ratios				Mean	Standard Error
	0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59		
Flush cut unpainted (III)	1/1	1/2	3/6	1/1	0.3929	0.0214
Flush cut painted (IV)	0/1	0/4	2/5	0/0	0.4246	0.2061
Shigo cut (V)	0/1	3/5	1/4	0/0	0.3749	0.1936
Total	1/3	4/11	6/15	1/1		

^a Numbers of infected trees among stem aspect ratios was not statistically significant, $P = 0.2578$.

Table 4. Diameters of pruning wound surfaces for the three pruning treatments.

Treatments (No.)	Range	Mean wound diameter (cm)	Standard Error
Flush cut unpainted (III)	4.1 – 17.0	7.8a	1.3568
Flush cut painted (IV)	4.8 – 8.1	6.6ab	0.3908
Shigo cut (V)	2.8 – 8.1	5.3b	0.5283

^a Mean wound diameters followed by different letters are significantly different, $P = 0.10$.

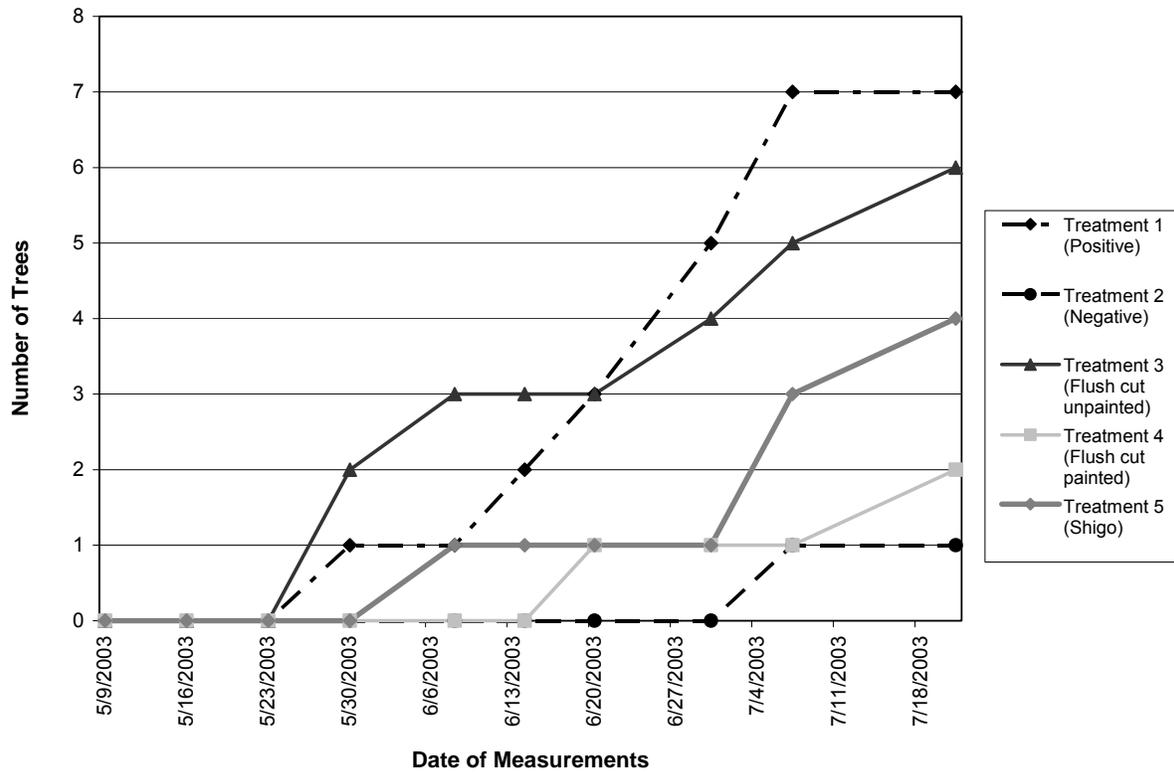


Figure 1. Symptom expression of *Quercus fusiformis* after inoculation with *Ceratocystis fagacearum* on April 30, 2003.

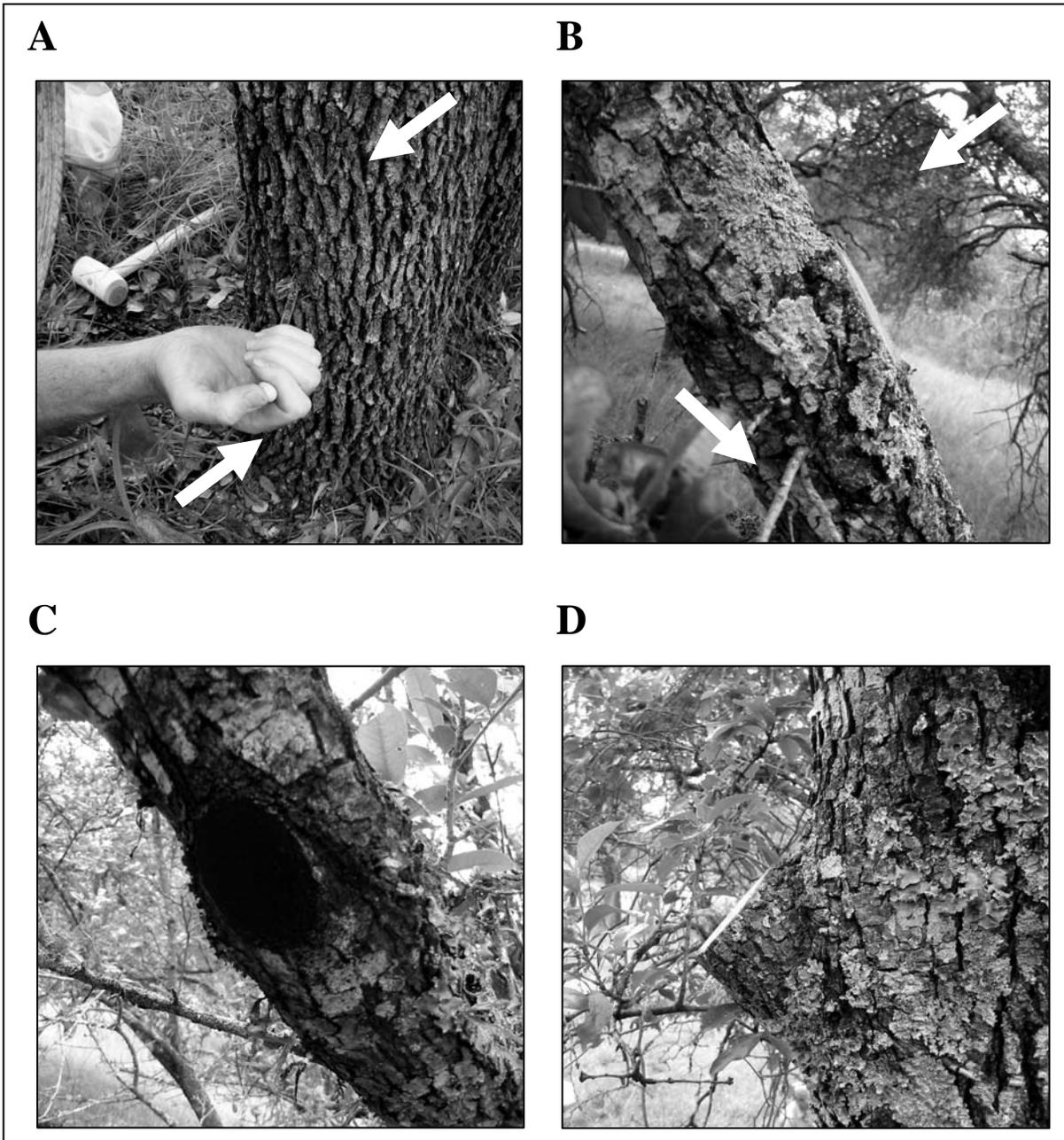


Figure 2. Treatment types for inoculation of *Q. fusiformis* with *C. fagacearum*. A = positive and negative (Treatments I and II), B = flush pruning cut, unpainted (Treatment III), C = flush pruning cut, painted (Treatment IV) and D = proper pruning cut (Treatment V). Arrows indicate location of wounds.

