The Proceedings of the 2nd National Oak Wilt Symposium

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PRELUDE

Fifteen years ago, in 1992, the first National Oak Wilt Symposium was held in Austin, TX. That symposium was held with a sense of urgency in the belief that valuable information concerning oak wilt would be lost without some attempt to document previous work on this destructive tree disease. Numerous older scientists who were key responders to the original oak wilt threat in North America were still available to summarize their contributions. There was also a need to review recent oak wilt research being conducted throughout the U.S. Several states were implementing management programs and there was a need to document their experiences in hopes of improving efforts everywhere. By all accounts, the first Symposium was a great success. Oak wilt, however, continues to be an important issue throughout many parts of the country. It was not a matter of if another oak wilt symposium would be held, but when.

The destructive impact of oak wilt in Texas still incites passion and determination to minimize losses of valuable trees. Nobody in Texas is more determined than members of the commercial arboricultural industry. Several arborists have been trying to raise interest in another oak wilt symposium for several years. Pat Wentworth, Russell Peters, Eugene Gehring and the leadership of the Texas Chapter of the International Society of Arboriculture were among those making the strongest case. Progressive education of their industry has always been an organizational objective of Texas arborists, and recent advances in oak wilt research were considered to be sufficient for another meeting. But this was not the only reason given as justification for another National Oak Wilt Symposium. Assessment and management tools are being refined, new technologies are being developed, and a new, growing membership in the arboricultural industry were all given as further reasons that another meeting would be well received. Following the formation of an organizing committee, a prospective speaker list was compiled with the intent to explore interest in the idea at the national level. We were delighted when every prospective speaker contacted expressed enthusiasm for the meeting and committed to participating. These Proceedings are the result of their efforts.

A successful symposium requires contributions from many dedicated individuals and organizations. We particularly want to thank the following people and their respective organizations: Members of the organizing committee: Patrick Wentworth, Austin Tree Specialists; Russell Peters, Arborilogical Services, Inc.; Eugene Gehring, Urban Renewal, Inc.; Dr. Damon Waitt, Lady Bird Johnson Wildflower Center; Dr. Thomas Greene, The Nature Conservancy; Dr. Michael Walterscheidt, International Society of Arboriculture, Texas Chapter; Dr. David Appel, Texas A&M University; and, from the Texas Forest Service, James Rooni, John Giedraitis, and Dr. Ronald Billings. We are particularly grateful to the International Society of Arboriculture, Texas Chapter, for sponsoring the Symposium and to the following cooperators: USDA Forest Service, Forest Health Protection, Lady Bird Johnson Wildflower Center, The Nature Conservancy of Texas, the Texas AgriLife Extension Service, and the Texas Forest Service. Finally, we thank the following companies for providing the resources to offer a first class venue: Bartlett Tree Experts, Mauget, Inc., Rainbow Technology, Inc., and Oak Wilt Specialists, Inc. More than 100 scientists, foresters, arborists, and concerned citizens attended the 2nd National Oak Wilt Symposium. We truly hope that the meeting met their expectations. It certainly met ours, and we hope that we have now set a precedent for future oak wilt symposia.

David Appel and Ronald Billings, Editors

INTRODUCTORY REMARKS

WELCOME TO TEXAS

James B. Hull
Texas State Forester
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Welcome to Texas - - - Y'all! We are honored to be hosting this prestigious symposium in Austin, and only 6 days after the 80th Texas Legislature adjourned after being in regular session for the past 140 days. This is a process that occurs every two years in Texas, whether we need it or not! Austin is not only the home of the State Capitol, but also the orange and white University of Texas Longhorns. However, the Texas Forest Service is part of The Texas A&M University System, 100 miles northeast of here in College Station. In honor of our school colors, the Aggies have just about perfected a way of genetically causing all of the Texas Bluebonnets in Austin to have a rich maroon color!

Texas is a mighty big and extremely diverse state: Vast Pineywoods of East Texas; rugged mountains of Southwest Texas; rich agriculture in the Northwest high plains; tropical citrus and other agriculture in far South Texas; and the beautiful rolling hill country in between. Texas has over 350 miles of shoreline along the Gulf of Mexico; 1200 miles of international border with Mexico and 250 miles of international border with Louisiana! It is 885 miles across Texas from north to south, and 835 miles across from East to West.

Texas has six major tree regions, totaling over 29 million acres of forest lands. Now that we have finished our second year of completing a statewide Forest Inventory and Analysis, we are finding that we might actually have up to twice as many acres of forest as previously thought. Commercial forestlands in Texas are undergoing tremendous restructuring in ownerships. Where forest industry historically owned and provided world class forest management on approximately one-third of these forest, recent divestiture of these lands has seen these forests now in the hands of TIMO's and REIT's. The other lands belong primarily to individual/family forest owners (64.8%) and public owners (8.0%).

Texas also has a tremendous acreage of range and other open space, yet at the same time we are becoming identified as an urban state. Texas has three of the nation's ten largest cities, and five of the top twenty. Of the 22.5 million Texas residents, 84 percent live in or near cities. One of the biggest challenges to Texas is the population growth into the rural/urban interface. As example, of the 32,000 wildfires that burned in Texas in 2005-2006, 85% of those started within 2 miles of a Texas community.

I was named Texas State Forester in 1996, and since then many folks will tell you that it has been one disaster after another. While some of these disasters have been of my doing, others have been associated with unprecedented wildfires, ice storms, hurricanes, floods, southern pine beetle outbreaks, wind storms, and the Texas Forest Service had the lead role in the Columbia space shuttle recovery efforts in 2003.

With changing roles and responsibilities, the Texas Forest Service has made the commitments to work smarter and provide the role of leadership in several areas of forestry. Currently, our staff is leading development of the Southern Critical Forestland Assessment for the 13 southern states. This is an effort to identify the forested areas that are in most need of priority attention. We recently completed the Southern Wildfire Risk Assessment that provides GIS tools to identify the highest areas of wildfire risk for planning, fire prevention, mitigation and response activities.

Another Texas-size disaster has been oak wilt devastation across vast areas of the Texas Hill Country in Central Texas. Dr. Ron Billings and his Forest Pest Management team have made significant progress over the past 20 years in dealing with this massive problem. To most successfully deal with oak wilt, we have effectively integrated and coordinated it with our Forest Stewardship, Urban, and FIA programs. I am very proud of these accomplishments in this area and no doubt you will hear more about it while you are in Austin.

Thanks again for coming to Texas. While you are here it is our goal to prove to you our claim that Texas is the friendliest state in the United States. Y'all come back now!

SECTION I

CURRENT UNDERSTANDING OF OAKS AND OAK WILT

AN OVERVIEW OF *QUERCUS*: CLASSIFICATION AND PHYLOGENETICS WITH COMMENTS ON DIFFERENCES IN WOOD ANATOMY

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ABSTRACT

The oaks (genus *Quercus*) are one of the most important groups of flowering plants and dominate large regions of the northern hemisphere. They are most prevalent in subtropical, temperate, and montane tropical regions. *Quercus* is phylogenetically divided into at least five major groups, of which three (the red oaks, white oaks, and intermediate oaks) are native to the New World. Overall, there are more than 200 species of oak in the Western Hemisphere, and probably a larger number in Asia, and relatively few in Europe. The center of diversity in the Americas is in the highlands of Mexico, with a secondary center in the southern United States. From the standpoint of susceptibility to disease, the phylogenetic groupings have some predictive capability, and in some cases this may be related to differences in ecology, physiology, and wood anatomy. White oaks in general are more diverse in the drier parts of North America, and have heartwood that is typically blocked by tyloses, while red oaks generally have fewer tyloses. Because tyloses block water flow through the heartwood, white oak wood makes good wine barrels while red oak wood does not. Given the greater susceptibility of red oaks to both oak wilt and sudden oak death (SOD), these differences in wood anatomy may be relevant.

Key words: *Ceratocystis fagacearum*, oaks, oak wilt

The oaks (*Quercus*) are among the most recognizable trees in the Northern Hemisphere, dominating large areas of North America, Europe, and Asia. They are also among the most economically-useful trees, providing high-quality lumber, firewood, tannins for leather, natural dyes, long-lived horticultural shade trees, wildlife habitat, animal feed (acorns), and even human food (acorns are still eaten in parts of Asia). Before the advent of steel-hulled ships, oak lumber was the primary material used in the construction of both merchant and warships in Europe and the Americas (and in fact, the hull of "Old Ironsides" is not iron, but oak covered with copper sheeting). Most botanists from the Northern Hemisphere are very familiar with *Quercus*. It is also well-known to the general populace, and is prominent in literature – often as a symbol of strength or character. There are many famous oak trees, including several “treaty oaks” in various parts of the U.S.

In the context of oak wilt, the purpose of this paper is to provide an overview of the genus *Quercus*, particularly in the Americas, with the goal of providing an entry into various aspects of the relationships of oaks and oak subgroups, information about the distribution and ecology of the genus, and discussion of some selected oak species groups (e.g., the "live" oaks) that are of particular interest in the context of oak wilt (caused by *Ceratocystis fagacearum* (Bretz) Hunt).

The genus *Quercus*, with probably more than 500 species worldwide, is placed in the family Fagaceae (Nixon 1989, 1993a, b, 1997a, b, c, Manos, Doyle and Nixon 1999), which includes the genera *Castanea* (chestnuts), *Chrysolepis* (California chinquapin), *Castanopsis*, *Lithocarpus*

(including tanoak), *Fagus* (the beeches), and the rare tropical *Trigonobalanus*, *Formanodendron*, and *Colombobalanus* (Nixon 1989, Nixon 2003); these three monotypic genera are sometimes lumped under *Trigonobalanus*). The oaks and beeches (*Fagus*) are wind-pollinated, while genera in subfamily Castaneoideae (*Castanea*, *Chrysolepis*, *Castanopsis*, and *Lithocarpus*) are probably all insect-pollinated. The remaining three "trigonobalanoid" genera (*Trigonobalanus*, *Formanodendron*, and *Colombobalanus*) are poorly known, and pollination is probably by insects in *Trigonobalanus*, but by wind in the other two genera. The fossil record of trigonobalanoids and castaneoids extends to the Oligocene of North America, as do verifiable *Quercus* fossils (Crepet and Nixon 1989 a, b, Nixon 1989).

Quercus is often considered to be a taxonomically difficult group. While it is true that interspecific hybridization is relatively common in *Quercus*, it is also true that many field botanists rely almost solely on characters of leaf shape to distinguish species of oak, although leaf shape and lobing are highly plastic and mostly unreliable as taxonomic characters (Nixon 1997b). When more fundamental characters such as twig and leaf pubescence, bud characteristics, and acorn morphology are used in combination with leaf shape characters, many specimens that might otherwise be labeled as hybrids are seen to be merely leaf forms of a particular species. This is particularly true in the cases of some white oak species such as *Q. stellata*, where botanists often erroneously dismiss specimens that lack the typical "cruciate" (cross-shaped) leaf form as hybrids with other species. These specimens, more often than not, are shade or juvenile-leaved forms of *Q. stellata*, which has a wide array of leaf shapes that deviate from strictly cruciate, although hybrids between this and other white oaks are well-known.

Hybridization between species in the same group is relatively common (e.g., it is easy to cross a white oak species with another white oak species). However, crosses between species from different groups (e.g., red and white oaks) are considered to be virtually impossible, although a few reports of such crosses exist (Cottam, Tucker and Santamour 1982).

QUERCUS in the AMERICAS

With more than 200 species in the Western Hemisphere (Nixon 1997b), *Quercus* is the most important genus of the family Fagaceae in terms of species diversity as well as ecological dominance. In the Americas, the genera *Fagus*, *Castanea*, *Lithocarpus*, *Chrysolepis*, and *Colombobalanus* have only 9 additional species compared to more than 200 species of *Quercus* (Nixon 2003). As such, *Quercus* is also the most important group of Fagaceae economically in the Americas.

Ecological Diversity

Quercus is found in an astonishing array of habitats ranging from tropical and subtropical to cold temperate climates. No other tree genus in the Northern Hemisphere has species in such a diverse array of habitats. Within these climatic categories, *Quercus* is found in tropical lowland forests, dry tropical forest, cloud forest, and various montane evergreen forests, including pine-oak, pine-fir, and relatively pure stands of evergreen oak. In subtropical regions, oak is often a component or dominant in chaparral, oak woodland, pine-oak, juniper-oak, and various other phases including both mediterranean (winter-rain) and monsoonal summer-rain areas. Both the temperate deciduous forests of eastern North America and Europe are dominated over large areas by species of oak, and in the southeastern U.S., these forests grade into subevergreen types

dominated by members of the live oak group. In many regions, such as central Texas and parts of southern California (Nixon 2002), oaks are the only large native trees in the landscape.

Oak Centers of Diversity

In the Western Hemisphere, Mexico has by far the largest number of oak species, especially in the three major mountain systems, known as the Sierra Madre Occidental, Sierra Madre Oriental, and Sierra Madre del Sur (Nixon 1993a). However, oaks also become dominant elements in the mountains of the northern deserts (Chihuahuan and Sonoran) above about 1800 meters elevation. The majority of oak species in Mexico are found in oak-conifer, oak forest, cloud forest, or chaparral habitats. Several oak species, mostly with broader distributions into Central America, occur at lower elevations on both coasts of Mexico, particularly in the "cloud forests" but also in some cases extending into tropical dry forest (e.g., *Quercus corrugata*, *Q. insignis*, *Q. elliptica*, and *Q. sapotifolia*).

In Central America, the number of oak species diminishes as one heads south. There are approximately 45 species recognized from the southernmost state of Mexico (Chiapas) to Panama. The greatest number of species in Central America is on the Pacific (drier) slope. A single species of oak occurs in Colombia (*Q. humboldtii*, a member of the red oak group). Oaks are not known in South America outside of Colombia, and probably arrived in northern South America from Central America relatively recently, probably during the Pleistocene.

Oaks are also dominant in forests in Asia, especially in subtropical/temperate China. Lesser centers of diversity are found in the southeastern United States and the Himalayan belt. Europe is actually relatively depauperate in terms of oak species, probably due in large part to past glaciation which likely decimated oak populations in northern Europe.

How Do You Tell an Oak?

Although in eastern North America and Europe, the typical lobed leaf of most oaks species is diagnostic, and recognizable by the general populace, throughout the range of *Quercus* the lobed leaf is not common. Only a few of the species found in Mexico, the subtropical Mediterranean region, and subtropical and tropical areas of Asia have lobed leaves. By far the most common leaf form in *Quercus* is an entire (neither lobed nor toothed) or regularly-toothed leaf without lobes. Thus, the acorn (a nut subtended by or enclosed by a hardened cup) is the most important diagnostic feature. Unfortunately, the genus *Lithocarpus* also has a similar acorn fruit, but for North America, there is just a single species of *Lithocarpus* (*L. densiflorus*, the "tanoak") in California and southern Oregon. In this case, *Quercus* is separated from *Lithocarpus* by the different form of the male catkins in the two genera, lax and hanging in *Quercus*, and upright in *Lithocarpus* (which is insect-, not wind-, pollinated). In summary, the genus *Quercus* is reliably recognized by the combination of the acorn and lax male catkins.

Some confusion persists about the term acorn. An acorn is technically the entire fruit of the oak, which is made up of both the cup and the single-seeded nut that it encloses. However, the nuts, after falling from the cup in the fall, are often referred to simply as acorns, which is technically (botanically) incorrect, but because of common usage must be considered an alternate, popular definition of acorn.

***Quercus* Subgroups**

The oaks are divided into two subgenera (Nixon 1993b, Manos, Doyle and Nixon 1999), subgenus *Cyclobalanopsis* (sometimes recognized as a separate genus), restricted to eastern

Asia, and subgenus *Quercus*, with the remainder of species, including all species native to North America and Europe. Within section *Quercus*, there are four recognized sections: Section *Cerris* (Europe, Mediterranean, Asia), Section *Lobatae* (red oaks – New World only), Section *Protobalanus* (southwestern U.S., northwestern Mexico), and Section *Quercus* (white oaks) in the Americas, Europe, and Asia.

Name Issues in *Quercus*

Although in North America the name *Quercus* is consistently and generally applied to oaks, there has been considerable confusion regarding subgeneric groupings of oak, both in terms of common names and scientific nomenclature. This is in large part due to vague designations of rank in some of the older literature, where often no distinction was made between the rank of subgenus and section below the level of genus (e.g., Trelease 1924). The problem is compounded by the use of various common names for different groupings. Thus, for the white oak group, one may see the names *Lepidobalanus* or *Leucobalanus*; because the type of the genus (*Quercus robur* L.) is a white oak, the white oak group is correctly referred to as Section *Quercus* (within Subgenus *Quercus*). Likewise, one may see the red oak group referred to as subgenus *Erythrobalanus*; based on recent molecular and morphological work, it is best recognized as a section with subgenus *Quercus*, and the correct name for the red oaks is then *Quercus* subgenus *Quercus* section *Lobatae* (Nixon 1993b). It also is worth noting here that the red oaks are sometimes also referred to as the black oaks, particularly in the western U.S., where the common eastern red oak (*Q. rubra*) does not occur naturally, and the common lobed-leaf red oak of California is *Q. kelloggii*, or California black oak.

Morphological Variation in Oaks

Along with incredible habitat variation, there is corresponding morphological variation in New World *Quercus*, particularly in leaf form. Most *Quercus* species, except for several from eastern North America, do not have lobed leaves – entire, toothed or spinescent leaves are more typical. Many of the montane tropical species, particularly in the red oak group, have similar, entire, glossy leaves, and the taxonomy of the tropical oaks remains problematic. This, along with hybridization and a lack of adequate fruiting material in collections, adds to the difficulty in understanding the taxonomy of these tropical groups, and these are perhaps the most difficult species in the genus.

Ecology of Red Oaks vs. White Oaks

In a very broad sense, based on numbers of species in various habitats, it is clear that white oaks occupy a greater range of habitats than do red oaks, particularly drier habitats. Thus, red oaks are less diverse in the dry regions of the southwestern U.S., and red oaks predominate in the wetter areas of Central America. That said, particular species of red oak may be more drought adapted than particular white oaks; such is the case with various red oak species from the drier phases of Mexican highlands.

The Significance of Wood Anatomy of Oaks

Species of the white oak group typically have smaller diameter vessel elements, that are thinner-walled and angular in outline, in contrast to the larger, round, thick-walled vessels of red oaks (Fig. 1). Tillson and Muller (1942) surveyed a large number of species, however, they found that many evergreen white oaks from the southwestern U.S. and Mexico had vessels resembling

those of red oaks, that were larger, thick-walled and rounded in outline. These included both *Q. fusiformis* and *Q. virginiana* in the live oak group. However, Tillson and Muller did not survey the occurrences of tyloses. In mature wood the heartwood of white oaks typically fills with tyloses (see Fig. 1). Tyloses are intrusions into the vessels of the heartwood that become lignified and impregnated with tannins, literally "plugging" the vessels (Fig. 1b). This not only reduces the rate of flow of water/sap in the heartwood, but also creates a mechanical (and chemical) barrier to the growth of some wood-infecting fungi.

Because red oaks have fewer tyloses in healthy mature wood, the wood is much more porous than that of white oaks, and red oak lumber is not as resistant to fungal decay and insect damage, nor is red oak suitable for construction of items that must hold water, including barrels, kegs, and ships. Indeed, certain white oaks such as *Q. stellata* (post oak), because of their decay-resistant wood, were preferred not only for fence posts but also for railroad ties and structural and support timbers in contact with ground or in lower portions of buildings. Until recently, oak flooring was almost entirely from white oak sources, due to its resistance to decay in humid climates. But recently, this has been largely replaced by flooring cut from faster-growing red oak species such as *Q. velutina* (black oak) and *Q. falcata* (southern red oak). Although it is clear that red oak lumber is much more susceptible to decay when in contact with the ground than is white oak lumber, this does not necessarily translate directly to fungal disease resistance in living plants. However, the general pattern of greater susceptibility of red oaks to oak wilt may be related at least in part to these wood-anatomical differences.

The Live Oak Group: ***Quercus virginiana*, *Q. fusiformis*, *Q. minima*, *Q. geminata*, *Q. brandegei*, and *Q. oleoides*.**

It is important to note that although many oaks in various regions are referred to as “live oaks,” some are not members of series *Virentes* (for example, the California live oak, *Q. agrifolia*, is a red oak species; see Nixon 2002). The discussion here will focus on only the phylogenetically-related group of live oaks centered around *Q. virginiana*. The live oak group is one of the dominant elements of the oak flora of the southeastern coast of the U.S., extending into central Texas, and in isolated pockets through Latin America (as *Q. oleoides*) as far south as Costa Rica.

***Quercus* series Virentes Trelease (1924): A Subgroup of the White Oaks**. Distinctive features: very drought tolerant. An unusual feature of the live oak group (shared with the Glaucoideae) is the occurrence of fused cotyledons in all species. On germination, the petiolar region of the cotyledons elongates as a cotyledonary tube, pushing the hypocotyl/epicotyl axis deep into the soil, sometimes as much as 15 cm. The adaptive significance of this feature appears to be both drought and fire tolerance, since the crown of the plant is buried deep under the soil and less likely to either desiccate or be damaged in a quick-burning fire. All of the live oak group also regenerate extensively after fires by root-sprouts, often forming thickets for the first years before trees become emergent; or in the case of *Q. fusiformis*, such clones eventually form copses ("shinneries") that are connected extensively by both rhizomes and root grafts. This, of course, is one of the major considerations in developing strategies in treating oak wilt in live oak in central Texas and elsewhere.

Q. virginiana (live oak): The most widespread and famous of the live oak group, *Q. virginiana* is found from Virginia to Florida, and westward along the coastal states into Texas. It forms distinctive evergreen woodlands usually on deeper, better soils. Live oak was an

important resource for shipbuilding in the 18th and 19th centuries, providing structural beams and framework. A typical leaf-form is illustrated in the herbarium specimen in Figure 2.

Q. fusiformis (Texas or plateau live oak): This species intergrades broadly with *Q. virginiana* in the areas between the Edwards Plateau and coastal Texas; material from Brazos County eastward is typical *Q. virginiana*, while material to the west and north is more typical of *Q. fusiformis*. Because of this broad zone of intergradation, some botanists prefer to lump *Q. fusiformis* as a variety of *Q. virginiana*. However, in its extreme forms in northern Mexico (e.g., in the mountains near Monterey, Nuevo Leon), *Q. fusiformis* is very distinctive with long, tapered acorns (not shown), and usually narrower more acute leaves (Fig. 3). In these features, *Q. fusif*ormis from northern Mexico approaches the morphology of *Q. brandegei* from Baja California. Based on the completely different habitat preferences and distinctness of the material from northern Mexico, I prefer to follow Muller and treat the two taxa as separate species with a broad zone of intergradation in central Texas (Fig. 4). This better reflects the very different ecological parameters that coincide with the two distributions, including far less rainfall and a distribution almost entirely on limestone in the range of *Q. fusiformis*.

Q. minima (dwarf live oak): This species is found only on deeper sands in the southeastern U.S. and forms extensive rhizomatous colonies, usually less than 1 meter tall. It is characterized by a tendency to produce two different leaf forms on the same stems, a "juvenile" leaf form toward the lower portion of the stem that is often irregularly toothed and asymmetrical, and usually more entire, less lop-side leaf on the upper portions of the stem. Unfortunately, sprouts and regenerating colonies of both *Q. geminata* and *Q. virginiana* can resemble populations of *Q. minima*, and there is much confusion in the identification of these species.

Q. geminata (sand live oak): *Q. geminata*, although placed by some taxonomists as a synonym or variety of *Q. virginiana*, is distinct in morphology, ecological distribution, and also has a later flowering time than the latter. It is identifiable by the narrow, revolute leaves with impressed venation (Fig. 5). It occurs on deep sands more or less with the same coastal distribution as *Q. virginiana*, which is typically found on better loam or poorly-drained clay soils. The later flowering time and different edaphic preference of *Q. geminata* probably helps to maintain its distinctness from *Q. virginiana*, and putative hybrids are relatively rare, although these are noticeable for example at the western limits of *Q. geminata* in the regions of Biloxi and Gulfport, Mississippi.

Q. brandegei: This species is endemic to the Cape Region of Baja California, Mexico, extending from lower pine-oak forest into very dry thorn scrub habitats. In morphology, it is similar to the extreme forms of *Q. fusiformis* found in northeastern Mexico, but has even longer, acute acorns and narrow, acute leaves.

Q. oleoides: This is the most geographically widespread species of the live oak group, extending from northeastern Mexico (Tamaulipas) to Costa Rica, but only found in relatively restricted populations at low elevations in very tropical localities, in a variety of soils from sand dunes to volcanic and seasonally-inundated ("savannah") clays. On the western end of Cuba, there is a population of live oak that has been called *Q. oleoides* var. *sagraeana*, and is the only known oak stand in the Caribbean. This population is highly variable and seems to combine features of both *Q. oleoides* and *Q. geminata*.

SUMMARY

Oaks are extremely diverse in habit and habitat. However, broad patterns of correlation between oak groups and ecological environmental parameters are apparent, including some generalities

about wood anatomy that are relevant to disease resistance and susceptibility. White oaks in general are more drought adapted and also have more tyloses in the vessels of mature wood. Both of these features may contribute to greater resistance to infection and/or the symptoms of oak wilt in white oaks. However, the live oak group, a subgroup within white oaks, is also susceptible to oak wilt as evidenced by the severe infections in central Texas. Thus, wood anatomy alone is not a sufficient predictor of susceptibility within the oaks. Even so, given the phylogenetic patterns of susceptibility within oak groups, it is likely that oak wilt could become a major problem in Latin America, where red oaks dominate high elevations and wetter forests from Mexico to Colombia. Extrapolating from wood anatomy (and assuming a correlation with susceptibility), other mostly Asian groups of *Quercus*, the *Cerris* and *Cyclobalanopsis* groups (which have in general a red-oak like wood), as well as the genus *Lithocarpus*, may also ultimately be at risk.

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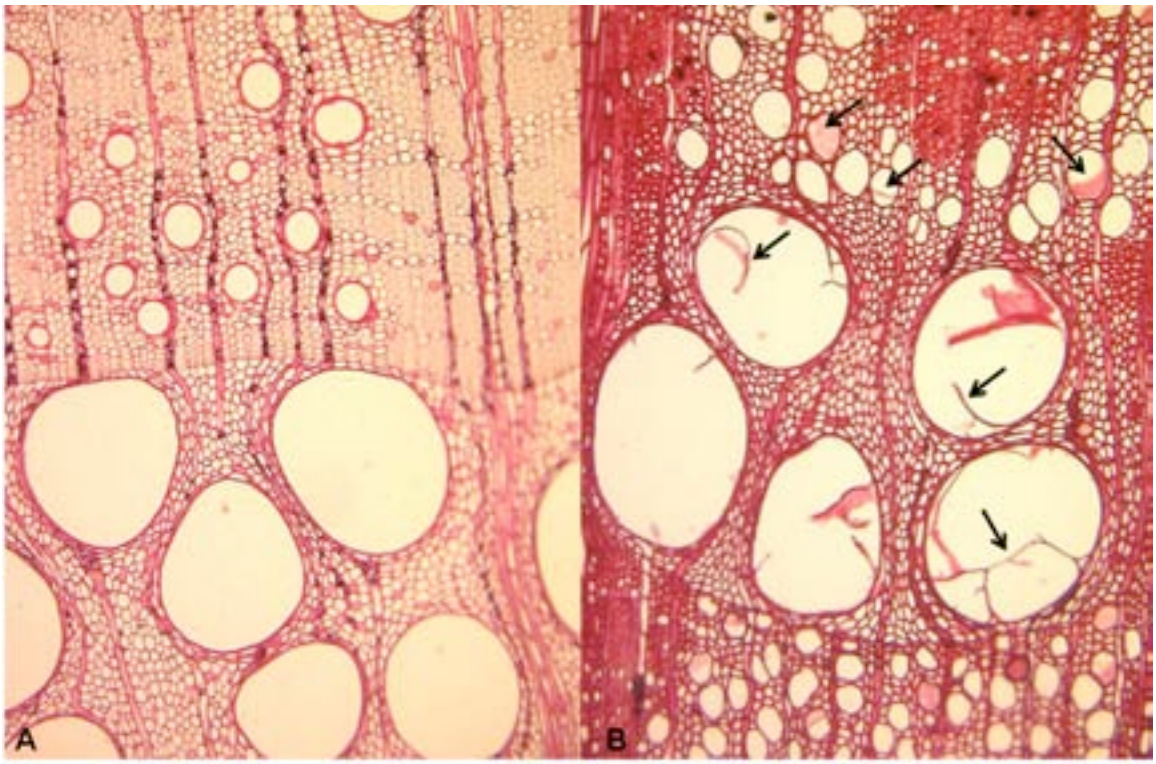

Figure 1. Standard light microscope preparation of cross sections of mature wood of *Quercus*. A. *Quercus rubra* with thicker-walled, more rounded vessels. B. *Quercus alba* with thinner-walled, more angular vessels in cross-section Note tyloses indicated by arrows in the large spring vessels and summer vessels of *Q. alba*, lacking in *Q. rubra*. (Magn. X).

Figure 2. Typical leaf form of *Quercus virginiana* in Florida (Muller 9830, BH).

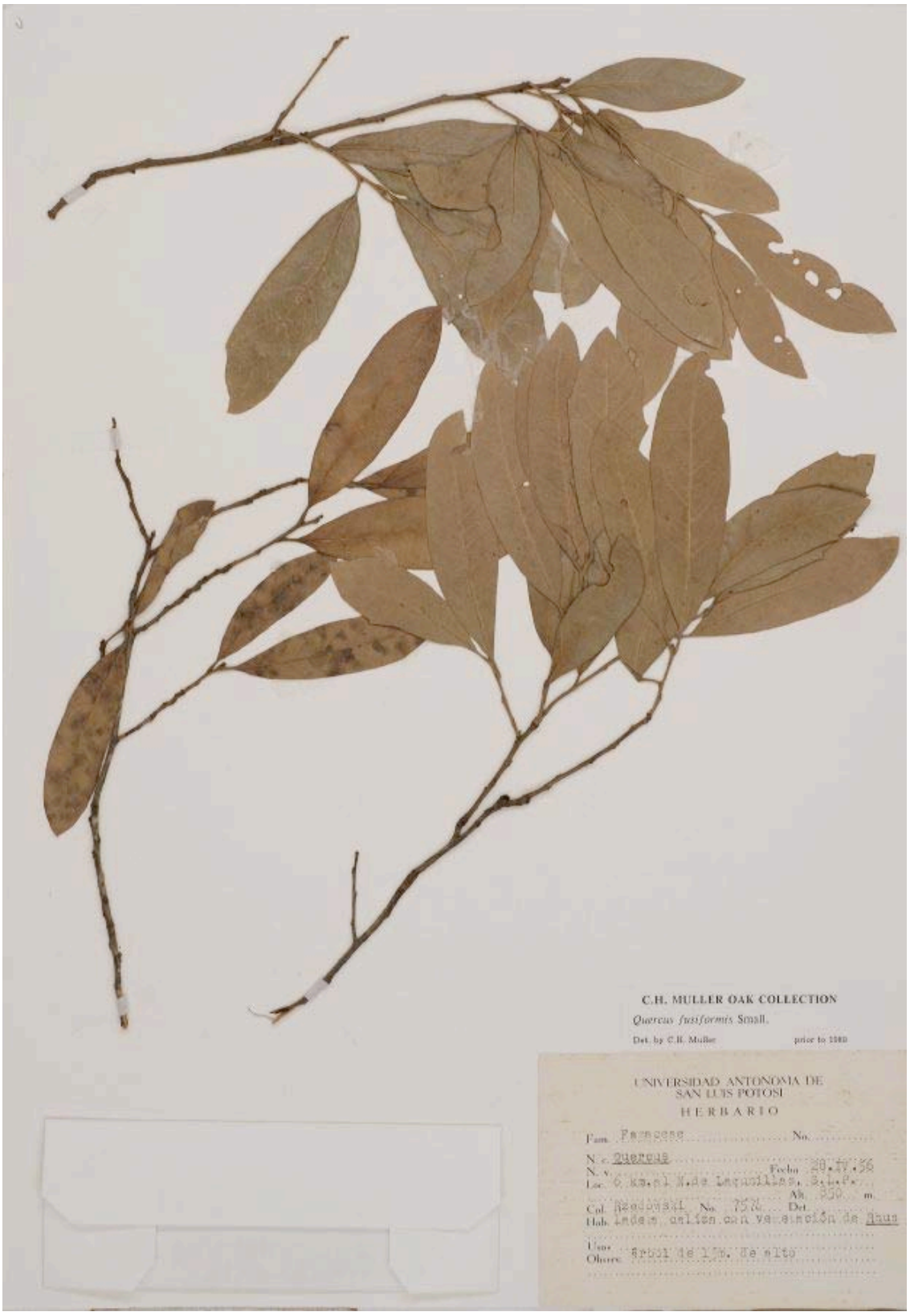

Figure 3. Typical leaf form of *Quercus fusiformis* in Mexico (Rzedowski 7574, BH).

Figure 4. Typical leaf form of *Quercus fusiformis* in Texas. (Dyal, Hazard and Fisher 152, BH).

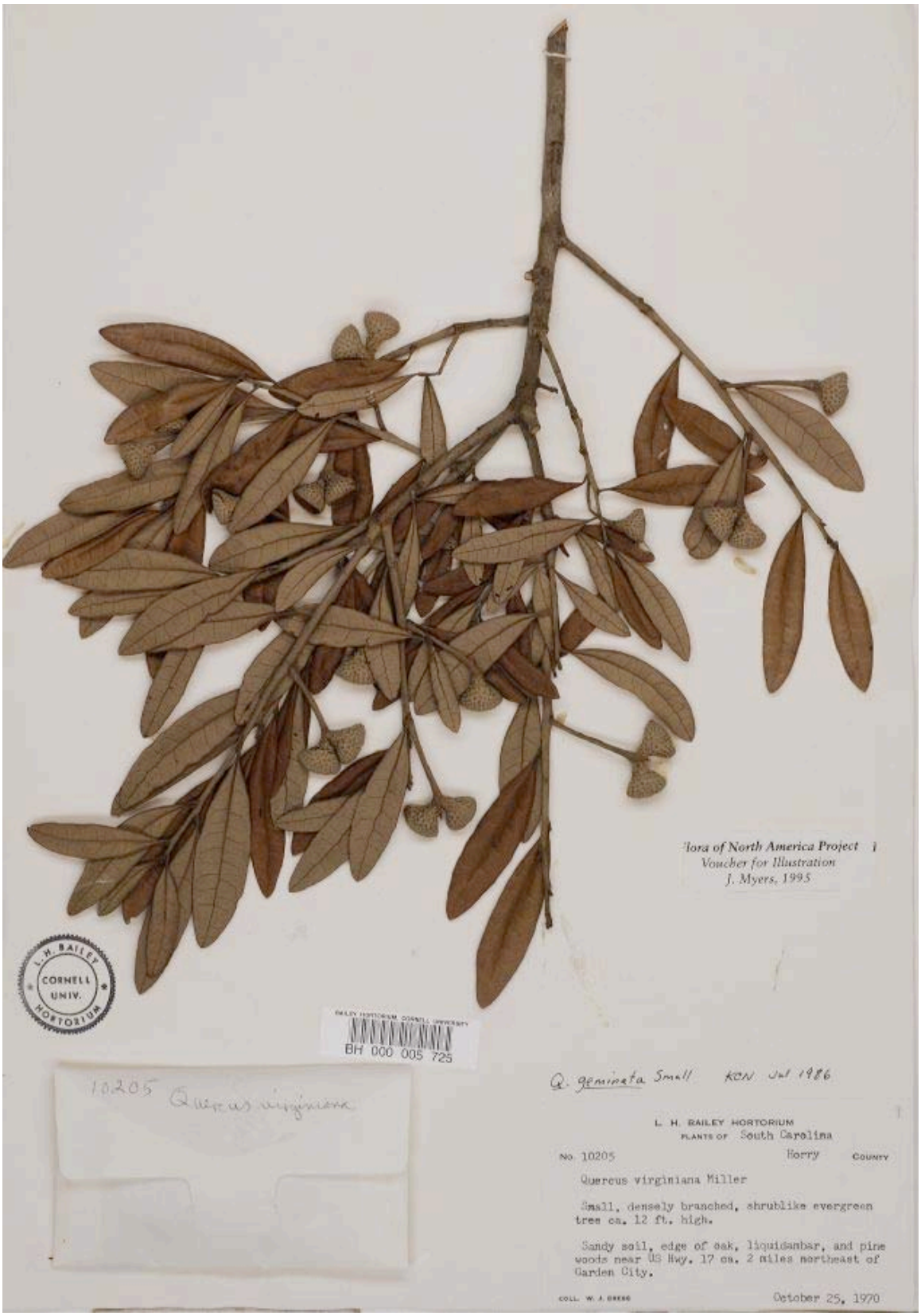

Figure 5. Typical leaf form of *Quercus geminata* (Dress 10205, BH).

THE GENUS *CERATOCYSTIS*: WHERE DOES THE OAK WILT FUNGUS FIT?

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ABSTRACT

Most species of *Ceratocystis* are plant pathogens, primarily colonizing sapwood near wounds on woody hosts, but only *C. fagacearum* causes a true vascular wilt. All species produce sexual spores in a sticky mass for insect dispersal, and most species, including *C. fagacearum*, produce fruity volatiles that are attractive to insects. Many *Ceratocystis* species produce sporulation mats on exposed wood, but only *C. fagacearum* forms pressure cushions or pads that push the bark away from the wood in order to crack the bark and expose the mats for fungal-feeding vectors (nitidulid beetles). Phylogenetic analyses of DNA sequences fail to identify a close relative of *C. fagacearum*. Limited genetic variation within *C. fagacearum* and the high susceptibility of some native oaks suggest that the fungus did not evolve in eastern U.S., but the evolutionary and geographic origins of *C. fagacearum* remain a mystery.

Key words: Evolution, fungal mats, genetic variation, taxonomy

Proper taxonomic placement of a species should say something about its biology. *Ceratocystis fagacearum* (Bretz) Hunt, the cause of oak wilt, is well placed in *Ceratocystis*, so we should not be surprised that it has insect vectors, produces fruity volatiles, and is a wound colonizer and vascular pathogen. Although its biology, morphology, and DNA sequences place *C. fagacearum* in the genus *Ceratocystis*, more precise placement within the genus has not proven possible, and there are several unique aspects to its biology. This paper reviews the genus *Ceratocystis* and discusses where *C. fagacearum* fits, or does not fit, based on phylogenetic analyses and biology. Genetic variation and possible origin of *C. fagacearum* also are discussed.

CERATOCYSTIS TAXONOMY AND EVOLUTION

The genus *Ceratocystis* once included the much larger genus *Ophiostoma* (Hunt 1956, Upadhyay 1981). The biology of *Ceratocystis* differs substantially from that of *Ophiostoma*, but they have converged on long-necked perithecia (sexual fruiting bodies) with sticky ascospore masses at their tip for insect dispersal (Harrington 1987). The two genera are not closely related and may have diverged more than 170 million years ago (Farrell et al. 2001). The genus *Ophiostoma* may be more than 85 million years old, near the time of radiation of coniferous bark beetles (Farrell et al. 2001, Harrington 2005), which are common vectors of *Ophiostoma* and related asexual genera, e.g., *Pesotum* and *Leptographium* (Harrington 1993). *Ceratocystis* may be younger than *Ophiostoma*, perhaps less than 40 million years (Farrell et al. 2001).

All *Ceratocystis* species have a Chalara-like endoconidial state, where the asexual spores are produced within deep-seated phialides. The genus name *Chalara* is restricted to the asexual state of another group of fungi, the discomycetes, but the genus name *Thielaviopsis* is available for the anamorphs of *Ceratocystis* (Paulin-Mahady, Harrington and McNew 2002). *Thielaviopsis* was

initially used to describe the two asexual states of *C. paradoxa*, the Chalara-like state and the aleurioconidial state. Aleurioconidia are thick-walled, chlamydospore-like, survival spores produced from specialized conidiophores. *C. fagacearum* does not form aleurioconidia but does form the endoconidial state (= *T. quercina*) (Paulin-Mahady, Harrington and McNew 2002).

Ceratocystis appears to be best placed with *Gondwanomyces*, *Petriella*, *Microascus*, and other members of the Microascales (Alexopoulos, Mims and Blackwell 1996), which also produce perithecia and sticky ascospore masses suitable for insect dispersal. Aside from *Ceratocystis*, Microascales are not plant pathogens; most are saprobes, and some are animal pathogens. The ancestor of *Ceratocystis* was probably a saprophytic species adapted to insect dispersal, and the ability to colonize wounds of living plants may have been crucial to its evolutionary success.

Phylogenetic analyses of ribosomal DNA sequences (Witthuhn et al. 1999, Paulin-Mahady, Harrington and McNew 2002) and DNA sequences of *MAT-2*, beta tubulin, and elongation factor-1 α (EF-1α) genes (Harrington, unpublished) show that there are at least four clades or complexes of species within *Ceratocystis* (Fig. 1). All but two species (*C. fagacearum* and *C. adiposa*) fall into four groups: 1) the *C. fimbriata* complex (Johnson, Harrington and Engelbrecht 2005), 2) the *C. paradoxa* complex (Paulin-Mahady, Harrington and McNew 2002), 3) the angiosperm and gymnosperm subclades of the *C. coerulescens* complex (Witthuhn et al. 2000a), and 4) the *C. moniliformis* complex (Van Wyk et al. 2006). Members of each of these clades share important ecological and morphological characters, such as ascospore morphology and presence or absence of aleurioconidia.

Four species of soil-borne pathogens with no known sexual state (*Thielaviopsis basicola*, *T. thielavioides*, *T. ovoidea*, and *T. populi*) are related to each other and appear related to *C. fimbriata* based on morphology and DNA sequences (Fig. 1) (Nag Raj and Kendrick 1975, Paulin-Mahady, Harrington and McNew 2002). These *Thielaviospsis* species, the *C. fimbriata* complex, and the *C. paradoxa* complex are joined by the common feature of aleurioconidia (Fig. 1). The unrelated *C. adiposa* and some Microascales also produce these survival spores, so it is hypothesized that the first *Ceratocystis* species produced aleurioconidia but that this character was lost in *C. fagacearum* and the *C. coerulescens* and *C. moniliformis* complexes.

Ceratocystis fagacearum and *C. adiposa* loosely group with the *C. monilformis* complex based on rDNA sequence analyses (Paulin-Mahady, Harrington and McNew 2002), but there is no statistical support for grouping these species using *MAT-2* sequences, beta-tubulin, or EF-1α (Fig. 1). Also, ascospores of *C. moniliformis*, *C. adiposa*, and *C. fagacearum* differ in shape (Hunt 1956).

Three *Ambrosiella* species that are symbionts with ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) have no known sexual state but are placed within *Ceratocystis* based on DNA sequence analyses (Fig. 1) (Cassar and Blackwell 1996, Paulin-Mahady, Harrington and McNew 2002). *Ambrosiella xylebori* and *A. hartigii* are closely related to each other but not to *A. ferruginea*, which is the nearest neighbor to *C. fagacearum*. However, the relationship of *C. fagacearum* and *A. ferruginea* is not well resolved. The relatedness of *C. fagacearum* to *Ambrosiella* species is intriguing, but it is unlikely that the oak wilt fungus evolved directly from a highly-specialized, asexual ambrosia beetle symbiont (Harrington 2005). However, *C. fagacearum* may share a *Ceratocystis* ancestor with an ambrosia beetle symbiont.

INSECT ASSOCIATIONS IN *CERATOCYSTIS*

Ceratocystis species typically form a mat of mycelium on the diseased host. Black perithecia with long necks position masses of ascospores above the mat. The ascospores are held together in a sticky, hydrophobic matrix, so the spores are not readily separated by water but instead have an affinity for the hydrophobic exoskeleton of insects. Wind and rain dispersal of conidia from such mats may occur, but the mats are more important as the sight for fungal feeding and acquisition of spores by insects (Moller and DeVay 1968). Insects must leave the mats and then visit wounds on susceptible plants for successful pathogen transmission.

Most *Ceratocystis* species produce fruity odors similar to that of banana, while *C. fagacearum* has an aroma described as "cantaloupe." These aromas are due to small chain fatty acids and esters, or fusel oils, which are thought to be attractants for fungal-feeding insects (Lin and Phelan 1992, Kile 1993). These compounds are toxic and may reduce grazing by insects that are not regular fungal feeders, thus leaving the mats for vectors, such as Nitidulidae (Coleoptera), which typically tolerate high concentrations of mycotoxins (Dowd 1995). Fusel oils may also be phytotoxic, though their role in pathogenesis is not clear (Kile 1993).

Fungal-feeding insects such as drosophilid flies (Diptera) and nitidulids have been frequently associated with mycelial mats, but most *Ceratocystis* species do not have specific insect vectors (Kile 1993). For instance, Verrall (1941) isolated a common hardwood-staining species, *C. moniliformis*, from ambrosia beetles and three other families of beetles. *C. variospora* and *C. populicola*, cause of almond canker and aspen canker, respectively, have been associated with nitidulids, but also with other insects (Moller and DeVay 1968, Hinds 1972). Various species of nitidulids have been shown to be vectors of *C. paradoxa* (Chang and Jensen 1974).

The association of nitidulids with the oak wilt fungus is particularly strong and may be due to the latter's capability of producing mats under the bark of freshly-killed trees. *C. fagacearum* forms pressure cushions or pads that push the bark away from the wood in order to crack the bark for insect access and form cavities for mat formation (Fergus and Stambaugh 1957, True et al. 1960, Gibbs and French 1980). No other *Ceratocystis* species is known to produce such pressure pads. Many species of nitidulids inhabit *C. fagacearum* mats, but *Carpophilus sayi* and *Colopterus truncatus* appear to be particularly important vectors in the Upper Midwest (Cease and Juzwik 2001, Juzwik, Skalbeck and Neuman 2004, Ambourn, Juzwik and Moon 2005).

Bark beetles are not common vectors of *Ceratocystis* species. Only four species (*C. smalleyi*, *C. laricicola*, *C. polonica*, and *C. rufipenni*) are known to be adapted to bark beetle vectors (Harrington and Wingfield 1998, Johnson, Harrington and Engelbrecht 2005). These species lack fruity odors, which would not be needed to attract vectors because the fungus sporulates in the bark beetle galleries. Fusel oils are toxic, and bark beetles may not survive well in galleries heavily colonized by *Ceratocystis* species producing these volatiles. Each of the bark beetle associates is homothallic (self-fertile), which would be important for sexual reproduction as there is not likely to be suitable movement of insects between beetle galleries to assure cross-fertilization. Also, conidium production is absent or rare in *Ceratocystis* species associated with bark beetles, presumably because there is no need for cross-fertilization.

Although there has been considerable debate about bark beetles as vectors of *C. fagacearum* (Gibbs and French 1980, Merrill and French 1995), comparisons with other *Ceratocystis* species suggest that the oak wilt pathogen is not well adapted to such a vector. Bark beetles lay eggs in trees weakened by oak wilt, but the next generation of beetles would not likely carry *C. fagacearum* propagules in high numbers or frequently introduce propagules into living branches. Trees killed by oak wilt tend to be dominated by one or the other of the two mating types of *C.*

fagacearum (Apple et al. 1985), and without an insect to cross-fertilize the mycelia, the fungus would not be able to produce fruiting bodies and ascospores in beetle galleries. Also, *C. fagacearum* produces toxic aromatic compounds, and mycelial mats would tend to plug the beetle galleries and suffocate larvae and teneral adults.

Bark beetles may be significant vectors in regions where nitidulids are less effective in carrying *C. fagacearum* (Rexrode and Jones 1970). In Europe, the bark beetle *Scolytus intricatus* has been suggested as a potential vector because it has a life history and behavior more suited to overland transmission than that of the bark beetles implicated as North American vectors, i.e., *Pseudopityophthorus* spp. (Webber and Gibbs 1989). However, it is questionable if *C. fagacearum* could become established in an ecosystem without suitable nitidulid vectors.

Only three species of *Ambrosiella* related to *Ceratocystis* have been described (Fig. 1), but ambrosia beetle symbionts are not well studied and there are probably many more relatives of *Ceratocystis* that serve as food for these highly specialized, xylem-inhabiting Scolytinae (Harrington 2005). Most ambrosia beetles have specific symbiotic fungi that colonize the wood and produce special spores or modified hyphal endings for insect grazing (Batra 1967, Beaver 1989). Many of the ambrosia beetles have special spore-carrying sacs, called mycangia, and the fungal symbionts are transported in these sacs (Batra 1963, Francke-Grosmann 1967, Beaver 1989). Glandular secretions into the mycangium facilitate yeast-like growth (Norris 1979).

Ceratocystis fagacearum has been isolated from mycangia of ambrosia beetles (Batra 1963), but it is unlikely that ambrosia beetles introduce *C. fagacearum* into living oaks. Still, an evolutionary link between *C. fagacearum* and ambrosia beetle symbionts is intriguing, and there should be further work on the associations of oak wilt and ambrosia beetles.

Another form of dispersal for *Ceratocystis* species is in ambrosia beetle frass. Members of the Latin American subclade of *C. fimbriata*, including *C. cacaofunesta* and *C. platani* (Harrington 2000, Ocasio, Tsopelas and Harrington 2007), and *T. australis* (Kile 1963) have been shown to be dispersed in frass when ambrosia beetles attack trees previously colonized by the pathogens. The sawdust and fungal propagules expelled from the trees as the adult beetles clean their tunnels may be dispersed by wind or rain splash for relatively short distances. The *C. fimbriata* species produce long-lived aleurioconidia in wood. The myrtle wilt pathogen, *T. australis*, does not produce aleurioconidia, but viable conidia and conidiophores are expelled by the insect tunneling. *C. fagacearum* was not isolated from frass expelled by ambrosia beetles attacking trees with oak wilt (Peplinski and Merrill 1974), but this dispersal mechanism needs further study.

DISEASES CAUSED BY *CERATOCYSTIS* SPECIES

Ceratocystis species grow mostly on woody angiosperms, and the *Ceratocystis* ancestor may have grown on a range of dicots. The *C. paradoxa* complex attacks monocots, especially palms, and a subclade of the *C. coerulescens* complex is found exclusively on gymnosperms (Fig. 1). Adaptations to these host groups may be derived characters. The non-aligned *C. adiposa* colonizes a wide range of hosts, sometimes as a saprophyte on conifer wood, but it also causes a root rot of sugarcane (Kile 1993). Colonizers of oaks and other Fagaceae are found in the *C. fimbriata*, *C. coerulescens* (angiosperm subclade), and *C. moniliformis* complexes. With the possible exception of soilborne pathogens, *Ceratocystis* and *Thielaviopsis* species are wound colonizers (Kile 1993), and in their native ecosystems most species appear to colonize only a limited area around the wound and are relatively benign pathogens.

Most economically-important diseases caused by *Ceratocystis* species are associated with a high incidence of wounding (Kile 1993). Ceratocystis wilt of cacao caused by *C. cacaofunesta* has been called "mal de machete" because of infection through machete wounds (Engelbrecht et al. 2007), and canker stain of plane tree caused by *C. platani* is also strongly associated with human-caused wounds (Engelbrecht et al. 2004, Ocasio, Tsopelas and Harrington 2007). In addition, these pathogens and the cause of Ceratocystis wilt of eucalyptus (*C. fimbriata sensu stricto*) can be transmitted in infected cuttings (Harrington 2000, Engelbrecht et al. 2007). *Quercus* species are not often propagated in this manner, and transmission of *C. fagacearum* in rooted cuttings is not likely.

Some *Ceratocystis* species colonize the host xylem far from the wound, but only *C. fagacearum* causes a true vascular wilt (Kile 1993). As *Ceratocystis* species colonize the sapwood of trees, they attack living parenchyma cells, inducing a dark discoloration of the xylem. In addition to colonizing sapwood, some *Ceratocystis* species cause cankers by killing the cambium and inner bark tissue (Kile 1993). These "sapstreak" or "canker stain" diseases differ from true vascular wilts, in which the pathogen moves systemically through the host in the non-living vessels and tracheids, at least in the early stages of colonization (Dimond 1970).

From an evolutionary perspective, the switch from a sapstreak to a true vascular wilt pathogen like *C. fagacearum* may have been simple. In the case of the saprophytic *Ophiostoma querci*, the experimental transfer of a single gene (the gene coding for cerato-ulmin, a hydrophobin) from *O. novo-ulmi* allowed *O. querci* to systemically colonize elm and cause vascular streaking and leaf symptoms typical of Dutch elm disease, a true vascular wilt disease (Del Sorbo et al. 2000). A related hydrophobin, cerato-platanin, has also been implicated as a pathogenicity factor for *C. platani* (Carresi et al. 2006), and phytotoxins have also been speculated as pathogenicity factors in oak wilt (Dimond 1970). Thus, one or a few introgressed or mutated genes may have made *C. fagacearum* a true vascular wilt pathogen.

Few tree diseases caused by *Ceratocystis* species result in significant mortality in native ecosystems. One possible exception is myrtle wilt, a sapstreak disease of *Nothofagus cunninghamii*, in which *T. australis* moves readily from tree-to-tree through functional root grafts (Kile 1993). In spite of the rapid spread of the pathogen through sapwood and rootwood of *N. cunninghamii*, myrtle wilt is believed to be an important player in the natural stand dynamics of these Australian forests. However, genetic evidence suggests that *T. australis* may not have evolved in this forest type (Harrington, Steimel and Kile 1998). *Ceratocystis platani* also spreads through functional root grafts and causes substantial mortality of planetree (*Platanus acerifolia*) in urban plantings. However, neither root-graft transmission nor substantial mortality of sycamore (*P. occidentalis*) has been noted in natural forest stands in eastern U.S., where the pathogen is indigenous (Engelbrecht et al. 2004).

Initiation of new disease centers is relatively rare in myrtle wilt and canker stain of planetree; many more trees are killed through root graft transmission than through wound colonization. This also is true with oak wilt (Appel 1995b). With time, one would expect that an oak ecosystem with such highly root-grafted and susceptible species like Texas live oaks (*Quercus virginiana* and *Q. fusiformis*) would shift to a forest type with more resistance to oak wilt and/or less root grafting.

It is noteworthy that few vascular wilt diseases of forest trees have been recognized and few (or none) of these are thought to be endemic (Sinclair, Lyon and Johnson 1987). Verticillium wilt of trees in the U.S. is exclusively a disease of urban and agricultural landscapes (Harrington and Cobb 1984). Dutch elm disease causes a vascular wilt on continents where *Ophiostoma*

novo-ulmi or *O. ulmi* has been introduced because there has not been sufficient selection pressure on American or European elms for the development of the level of resistance found in Asian elms (Brasier 2001). Species of persimmon (*Diospyros* spp.) in the southeastern U.S., likewise, lack the resistance of Asian species to persimmon wilt, caused by *Acremonium diospyri*, a likely exotic vascular wilt pathogen (Sinclair, Lyon and Johnson 1987). The extreme susceptibility of many eastern North America oak species to oak wilt argues that these oaks did not evolve with *C. fagacearum.*

GENETIC VARIATION IN *CERATOCYSTIS*

Most species of ascomycetes are heterothallic, meaning that they can reproduce sexually only if two strains of opposite mating type come in contact. The MAT-1 and MAT-2 mating types are determined by different genes at the mating type locus. Homothallic species usually have both *MAT-1* and *MAT-2* genes at the mating type locus and thus have all the genes necessary for sexual reproduction without mating. There is a surprising amount of homothallism in the genus *Ceratocystis*, perhaps because of the unreliability of insect dispersal of conidia for cross-fertilization of mycelia. All known species in the *C. fimbriata* complex, all species in the gymnosperm subclade of the *C. coerulescens* complex, and *C. virescens* are homothallic through unidirectional mating type switching (Harrington and McNew 1997, Witthuhn et al. 2000b). Homothallism is also found in some species in the *C. paradoxa* and *C. moniliformis* complexes, and in *C. adiposa*, but the genetic basis of homothallism in these species is unknown.

In the heterothallic *C. fagacearum*, developing ascogonia on mycelial mats of one mating type are fertilized by conidia of the opposite type via insects from other mats (Hepting, Toole and Boyce 1952, True et al. 1960). The importance of sexual reproduction and ascospores in the epidemiology of oak wilt is supported by the fact that the two mating types occur in nature in roughly equal proportions (Yount 1954, Appel, Drees and Johnson 1985).

Natural populations of *Ceratocystis* spp. have considerable genetic variation, and introduced populations have very limited variation due to a genetic bottleneck associated with the founding of the population by a single strain or a few strains. The question of whether *C. fagacearum* is native to a portion of its known range within the USA or if it was introduced from some other region can be addressed by studying genetic variation in the pathogen, as has been done with *C. albofundus* (Roux et al. 2001), *C. platani* (Engelbrecht et al. 2004, Ocasio, Tsopelas and Harrington 2007), and *C. cacaofunesta* (Engelbrecht et al. 2007). In these three homothallic species, there is substantial genetic variation where the pathogens are native, and the populations are essentially clonal where they have been introduced. A natural population of a heterothallic species like *C. fagacearum* should have substantial genetic variation, as was found for the heterothallic *C. eucalypti* in Australia (Harrington, Steimel and Kile 1998).

Kurdyla et al. (1995) found surprisingly little variation among isolates of *C. fagacearum* using restriction fragment length polymorphisms (RFLPs) of mitochondrial and nuclear DNA. The 27 isolates obtained from throughout the known range of the species (mostly from Texas, but also from West Virginia and Wisconsin) showed no variation in the mitochondrial DNA markers. There was some limited RFLP variation among nine isolates using anonymous nuclear DNA probes, but the variation was substantially less than that found with similar markers in introduced populations of other out-crossing pathogens (Milgroom and Lipari 1993).

Mitochondrial DNA markers that were used with the *Ceratocystis* species mentioned above were applied to 37 isolates of *C. fagacearum* from Iowa, six from Minnesota, and one from Illinois (Harrington, unpublished). The mitochondrial RFLP polymorphisms were identified

using *Hae*III digestion of genomic DNA (Wingfield, Harrington and Steimel 1996). There were 24 scorable bands, and surprisingly, the 44 isolates had the identical banding pattern, except that one isolate from Iowa had an extra band of 2.4 kb (Fig. 2A).

Nuclear DNA fingerprinting was applied to the same 44 isolates (Harrington unpublished) by probing *Pst*I-digested genomic DNA with the oligonucleotide $(CAT)_5$ (DeScenzo and Harrington 1994). Out of 35 $(CAT)_5$ bands, only two were polymorphic; one band of 2.7 kb was present or absent, and another band was polymorphic, with one of four different bands (alleles) ranging in size from 2.8-2.9 kb present in each of the isolates (Fig. 2B). The level of variation found in *C. fagacearum* was dramatically less than that found in the heterothallic *C. eucalypti* (Harrington, Steimel and Kile 1998) and substantially less than in natural populations of the homothallic species. The low level of variation found in *C. fagacearum* was comparable to that of the introduced populations of homothallic *Ceratocystis* species and the putatively indigenous population of the asexual *T. australis* (Harrington, Steimel and Kile 1998).

Mitochondrial DNA in *Ceratocystis* species is inherited maternally (Harrington, Steimel and Kile 1998), so the mitochondrial DNA of the progeny in an ascospore mass is identical to the parental strain that produced the fruiting body. In contrast, the ascospore progeny from a fruiting body of a heterothallic species should have variation in nuclear DNA markers because the alleles of each parent would be recombined through meiosis. The essentially clonal nature of the mitochondrial genome of *C. fagacearum* in eastern North America and the limited variation found in the nuclear genome suggest that U.S. populations were derived from a single sporulating mat.

THE ORIGIN OF *CERATOCYSTIS FAGACEARUM*

There is little agreement on whether or not *C. fagacearum* is native to eastern North America. The pathogen was first reported in the Upper Mississippi River Valley in 1944, and it may have spread from there to the Appalachian Mountains (True et al. 1960, McDonald 1995). However, the fungus was likely killing oak trees in the Upper Midwest in the late 1800s (Gibbs and French 1980) and may have been present in Texas since the 1930s (Appel 1995b). The fact that the pathogen readily colonizes and kills many oak species in eastern North America supports the argument that *C. fagacearum* evolved elsewhere. Unfortunately, we have no close relative that could be used as a point of reference to surmise a continent of origin for *C. fagacearum*. Nonetheless, portions of the world with oak forests would be a good place to start.

Quercus and other potential host genera in the Fagaceae are distributed widely throughout the Northern Hemisphere, and the related *Nothofagus* is found in the Southern Hemisphere. The susceptibility of European and other exotic oaks has been demonstrated (MacDonald et al. 2001), and Chinese chestnut (*Castanea mollisima*) is also highly susceptible, suggesting that *C. fagacearum* is not a natural component of Eurasian forest ecosystems. Furthermore, no near relatives of *C. fagacearum* have been identified in either Europe or eastern Asia, where the mycoflora of oaks has been extensively studied.

Western U.S. and Canada also have a number of *Quercus* species, but the *Ceratocystis* species on these hosts are reasonably well-characterized, and none are morphologically or genetically close to *C. fagacearum*. However, *C. fagacearum* may be native to Mexico, Central America, or northern South America because the *Ceratocystis* species there, other than the agriculturally-important species, are not well known. Many species of oak occur in these regions, especially in cool, high elevation cloud forests (Ingens-Moller 1955), and it is possible that *C. fagacearum* is a wound colonizer of relatively resistant oaks there.

Even if a Latin American origin of *C. fagacearum* was accepted, the pathway of its arrival is difficult to envision. Introduction from another continent by human activity would be unlikely because mycelial mats form under bark and only when the sapwood and inner bark tissue are very moist (Gibbs and French 1980). Movement of oak logs from another region to the Upper Midwest in the late 1800s, if such shipments occurred, would probably have taken too long for the logs to arrive with fresh mycelial mats for nitidulid dispersal.

Another pathway could have been a spore-laden insect blown into the U.S. by a hurricane or other storm event. Bark beetles contaminated with *C. fagacearum* would likely be contaminated with only conidia, and the genetic data suggest that the introduction of *C. fagacearum* was via an ascospore mass. A storm-dispersed nitidulid beetle could have been contaminated with a single ascospore mass, but it would have had to visit a fresh wound on a susceptible oak tree in order to establish the pathogen. The possibility that *C. fagacearum* was established in eastern North America via an insect or group of insects from a single mycelial mat cannot be discounted, but an animal vector capable of wounding oaks should also be considered.

Birds have been discussed as potential vectors of *C. fagacearum*, but they have not been thought to efficiently or frequently carry the fungus and establish new infections (True et al. 1960, Gibbs and French 1980). However, a bird may have been responsible for the hypothesized single event that brought *C. fagacearum* to the eastern U.S. Sapsuckers (*Sphyrapicus* spp.), for instance, will remove bark to forage for insects (Walters, Miller and Lowther 2002), so they may rarely feed on insects on oak wilt mats and acquire spores on their beak. They also drill through the bark of healthy trees to produce sap, which attracts insects, and sapsuckers will consume inner bark and cambium tissues, thus potentially introducing the fungus into a suitable wound. The yellow-bellied sapsucker (*S. varius*) is migratory, overwintering in Central America and Mexico and migrating north to the Upper Midwest in the spring, the right season for infection.

CONCLUSIONS

The oak wilt pathogen is a typical member of the genus *Ceratocystis*, but there are several unique and noteworthy aspects to its biology. Phylogenetic analyses have failed to identify a close relative, though there is some relation to an ambrosia beetle symbiont. Like *C. fagacearum*, many *Ceratocystis* species form sporulation mats that emanate fruity odors, presumably to attract their fungal-feeding vectors, and nitidulids have been shown to be vectors of other *Ceratocystis* species. However, only *C. fagacearum* is known to form pressure pads on mats. Though other insect vectors may have importance in some regions, *C. fagacearum* does not have the adaptations found in other species of *Ceratocystis* that have bark beetle vectors.

Ceratocystis contains mostly plant pathogens that are wound colonizers, but only *C. fagacearum* causes a true vascular wilt disease. The high susceptibility of *Quercus* species in eastern North America to oak wilt suggests that *C. fagacearum* did not evolve here. Genetic data also indicate that the fungus has been introduced, perhaps as a single ascospore mass. Humans may not have been the agent of introduction, however. Instead, a storm-blown insect or migrating bird may have brought the pathogen from Mexico or Central America.

Ceratocystis fagacearum is now well established and causes substantial mortality of oak in some regions, especially in the Upper Midwest and parts of Texas. Suitable nitidulid populations, abundant mycelial mats and wounds, and root-grafted and highly susceptible oaks appear to be major contributors to the relative importance of oak wilt in these regions. If the pathogen is a relatively recent arrival, we might see it expand its range to similar oak forests.

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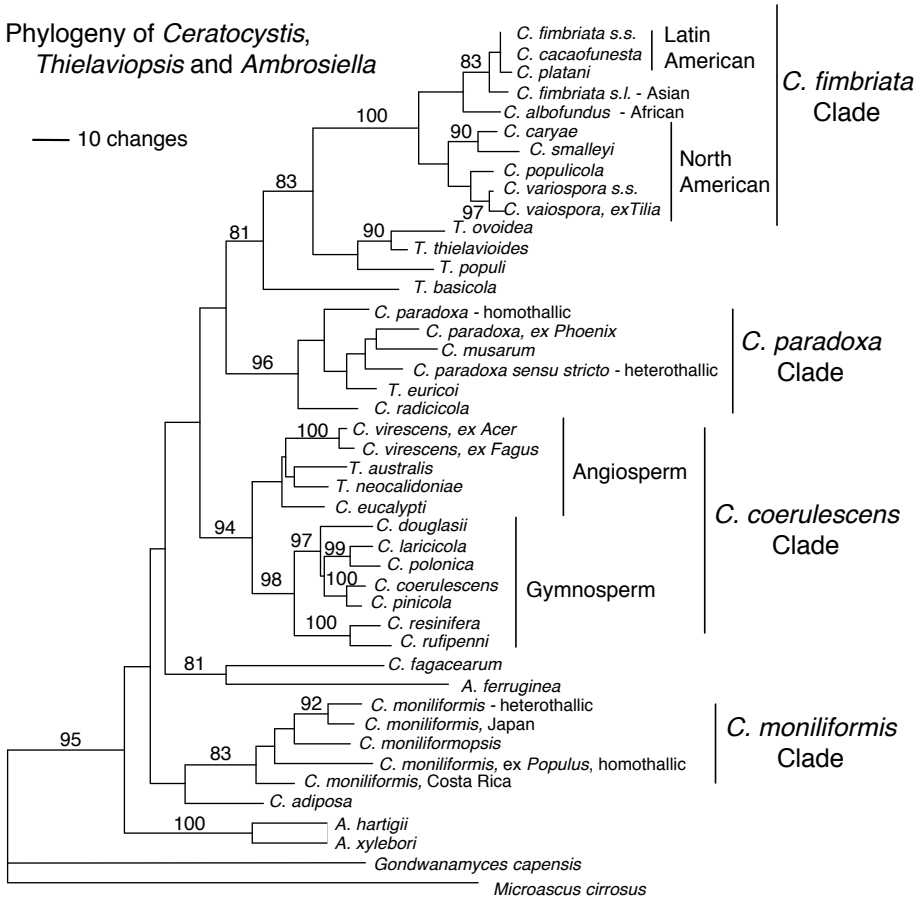

Figure 1. One of four most parsimonious trees of 1139 steps based on the DNA sequence of a portion of the elongation factor-1 α gene for *Ceratocystis*, *Thielaviopsis*, and *Ambrosiella* species. Of 1844 total aligned characters, 632 had to be eliminated from intron regions because of ambiguous alignment, 818characters were constant, 138 characters were parsimony un-informative, and 256 characters were parsimony informative. Gaps were treated as a fifth character. The consistency index was 0.3618 and the retention index was 0.6935. The tree was rooted to *Microascus cirrosus*. Bootstrap values (from 1000 replications) greater than 80% are shown above branches.

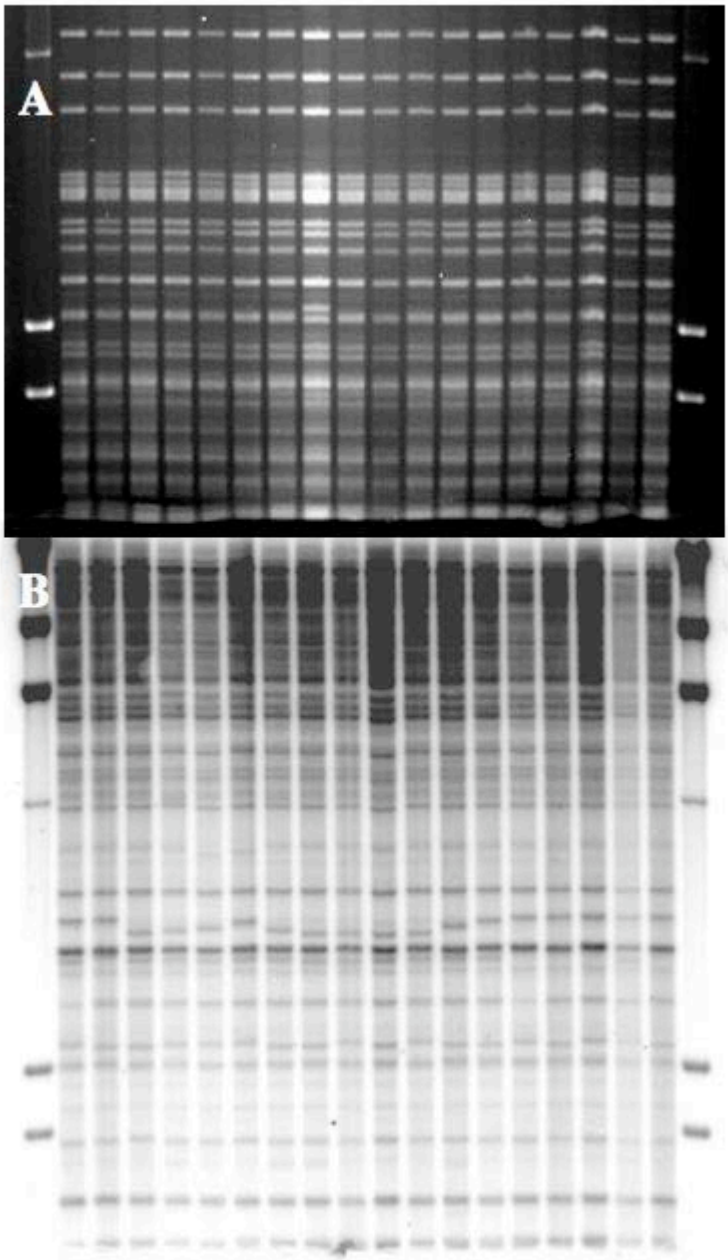

Figure 2. Mitochondrial (A) and nuclear (B) DNA fingerprints of *Ceratocystis fagacearum* isolates from Iowa. The markers in the outer lanes of A are 2.0, 2.3 and 4.3 kb from bottom to top. The markers in the outer lanes of B are 2.0, 2.3, 4.3 (faint), 6.5, 9.4, and 23 kb from bottom to top.

OAK WILT BIOLOGY, IMPACT, AND HOST PATHOGEN RELATIONSHIPS: A TEXAS PERSPECTIVE

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ABSTRACT

Oak wilt, caused by *Ceratocystis fagacearum*, continues to be a significant issue for natural resource managers, ranchers, and homeowners in Texas. However, the impact of the disease in central Texas is difficult to quantify. Several efforts have been made to assess the risk of oak wilt and illustrate the consequences of the disease. These surveys clearly show the disease remains at epidemic levels in many areas. The reasons for the high epidemic levels in central Texas, relative to the impact of oak wilt in other parts of the range in the U.S. are not entirely clear, but certainly the dominance of live oak is an important factor. The success of *C. fagacearum* in the oak juniper woodlands illustrates the adaptability of the pathogen to different *Quercus* spp. growing under presumably inhospitable environmental conditions. In spite of the high level of disease, oak wilt control is regularly and successfully implemented in Texas. Yet further studies are needed to gain a better understanding of this enigmatic pathogen and improve our ability to manage the disease to prevent further catastrophic losses.

Key words: *Ceratocystis fagacearum*, disease cycle, live oak, *Quercus fusiformis*

The geographic range of oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has changed little in Texas over the past 15 years since the first National Oak Wilt Symposium (see website http://www.texasoakwilt.org). A small number of counties have been added, but they do not represent any significant expansion of the disease (Fig. 1). Nonetheless, oak wilt continues to be a significant problem for homeowners, landowners, and natural resource managers in Texas. Current surveys have shown the disease is present in extremely high levels, with little evidence that these localized epidemics are decreasing. In addition, epidemics have erupted in new locations within the range that were previously free of the disease.

Oak wilt extracts a heavy toll on the central Texas landscape through a variety of manners. As has always been the case, the huge losses in the numbers of trees have a detrimental impact on property values. Losses are not only measured in terms of dollars, however. Trees in Texas represent a connection to the past and are an attachment to the natural world. The mortality of valuable shade trees, historic trees, featured landscape specimens, or any oak has negative consequences for how people view their environment. Oak wilt also has an indirect impact on other ecosystem components that depend on the central Texas oak savannah for existence. The best understood of these components is the golden cheeked warbler, an endangered songbird with a unique dependency on certain attributes of the juniper-oak woodlands that are common to the region.

The key to successfully controlling oak wilt has always depended on understanding the disease cycle. Disease cycles represent life cycles of pathogens and how they interpose on those of their hosts. For oak wilt, key features of the disease cycle were initially described by a large

number of forest entomologists and pathologists working in the 1950s (Gibbs and French 1980, MacDonald and Hindal 1981). Those descriptions were sufficient to construct a management program for much of the range of oak wilt (O'Brien et al 2000). The discovery of widespread oak wilt in Texas opened a new chapter in oak wilt management. Differences in conditions between Texas and other states with oak wilt required additional research to improve disease control (Appel 1995). As a result of this work, oak wilt can be successfully controlled under most conditions where the disease occurs in Texas.

HOW MUCH OAK WILT IS THERE IN TEXAS?

Disease Incidence at the Local Level

We are often confronted with the question, "How many trees are killed by oak wilt in Texas?" The number is undoubtedly enormous, but it is difficult to quantify with any accuracy. Several approaches have been used to map and quantify oak wilt in Texas, but these are usually far too narrow to get a complete picture of the scope of the mortality. On a narrow scale, a survey was conducted in 2001 on the Fort Hood Military Installation in an attempt to quantify the impact of oak wilt on the live oak population. The survey area was 119,000 ha. and located approximately 160 km. south of Dallas-Fort Worth, Fort Hood is located largely in Coryell County where oak wilt is considered to be a common feature in the landscape. The survey utilized IKONOS 1-meter satellite imagery to photo interpret potential oak mortality (Fig. 2). These mapped mortality centers were then transferred to Orthophoto Quarter Quadrangles (DOQQs) in order to ground truth the photo interpretation efforts. There were 1,164 polygons interpreted as diseased oaks, and 119 randomly sampled polygons were visited for ground diagnosis (Fig. 3).

Of the 119 mortality centers identified on the aerial photography, 23 were brush piles resulting from the systematic removal of junipers (*Juniperus ashei*) (Table 1). The remaining 96 polygons consisted of dying oaks, of which 82 were clearly caused by oak wilt. By extrapolating from these figures for the Fort Hood survey, there are potentially more than 800 actively expanding oak wilt centers, or one disease center every 150 ha.

In another approach, geographic information systems were used to assess oak wilt and communicate the threat of the disease to homeowners in Dallas, TX. Disease centers in a 129.5 km^2 (50 mi^2) block on the north side of Dallas were originally located with the assistance of urban foresters, arborists, and landscape managers. The disease centers were delineated and a 50 m zone around the center was surveyed for tree condition and species composition. Several analyses were conducted to determine the influence of numerous factors on the incidence and severity of the disease. The spatial distribution of the disease centers was analyzed to test for spatial dependency and randomness in their occurrence. As a result of these tests, maps were generated to illustrate the risk of oak wilt assuming spread rates of 1.6 km/year (1 mi/year) by insect vectors and 50 m/yr (162 ft/year) by root connections. These maps dramatically illustrate why there is a critical need to implement control measures in a valuable urban forest (Fig. 4).

Regional Disease Incidence

An additional indicator of the intensity of oak mortality in Texas caused by oak wilt can be found at the website http://www.texasoakwilt.org/. This website is an excellent source of information concerning all aspects of oak wilt, and contains an Internet Map Server that can be used interactively to view the locations of disease centers throughout many parts of the oak wilt range in Texas. There are several locations in the state where oak mortality dominates the

landscape, such as the view of Kerr, Gillespie, and Kendall counties (Fig. 5). Although not a complete documentation, there is sufficient survey data to illustrate the incidence and severity of oak wilt in the central Texas woodlands.

By any measure, it is apparent that oak wilt at many places is having a lasting impact on the ecology of the oak-juniper savannahs. The disease is also causing urban foresters and homeowners to question the value of widespread planting of live oak, the most popular shade tree in the state.

WHY IS IT SO BAD IN CENTRAL TEXAS?

The course of an oak wilt epidemic is very much a product of the host species present and the structure of the tree stand. These factors can contribute to a circumstance where the pathogen spreads rapidly, killing countless trees. The disease then presents unique challenges in successfully being contained and controlled. Just such a disastrous scenario has arisen in central Texas, particularly when compared to oak wilt where it occurs in the mid-Atlantic, Midwestern and North Central states.

Host Influences

Oak species vary widely in their response to infection by *C. fagacearum*. This variation was noted soon after the pathogen was first described (Henry et al. 1944), and extends to mortality rates, symptom development, and potential production of inoculum. All members of the red oak group (subgenus *Erythrobalanus*) have repeatedly proven to be susceptible to the pathogen (Fig. 6). In contrast, members of the white oak group (subgenus *Leucobalanus*) are very resistant to the disease with little or no loss of crown to dieback (Rexrode and Lincoln 1965, MacDonald and Hindal 1980). Live oaks (subgenus *Leucobalanus*) are intermediate between these two extremes in their response to the pathogen (Appel 1986). Most infected live oaks either die or lose large proportions of their crowns to the disease. About 15% or the infected live oak population may survive intact or respond minimally with little dieback. Foliar symptoms of oak wilt in live oak are distinct, often exhibiting a striking chlorosis and necrosis of the veins (Fig. 7). Foliar symptoms in red oak are most often described as a bronzing of leaves with an accompanying marginal scorch. The foliar response of white oaks is similar to that of the red oak group.

The Disease Cycle

The primary vectors of the pathogen are sap-feeding nitidulid beetles (Coleoptera: Nitidulidae). Insect spread of the oak wilt pathogen is sometimes referred to as overland, or long distance spread. The *C. fagacearum* vector relationship is not a simple one and may be one reason for the limited losses from oak wilt relative to other notorious tree diseases such as Dutch elm disease and chestnut blight. The only known source of inoculum for acquisition by nitidulids forms underneath the bark on the surface of the sapwood (Fig. 8). As the tree dies from oak wilt, the pathogen undergoes a brief period of saprophytic growth and forms a mat of growth on which spores are formed. This mat pushes the bark outward to make cracks for the nitidulids to visit and become contaminated with the spores. The mats range from a few to several inches long, usually have an elliptical shape, and emit a very sweet smelling odor to attract insects. In addition to providing spores for long distance transmission to initiate new disease centers in healthy trees, the mats allow for cross fertilization of the two mating types of the pathogen and

subsequent sexual reproduction. For some unknown reason, fungal mats only form on diseased red oaks. They do not occur on infected white oaks.

In addition to the fungal mats, nitidulids require a wound on the target tree in order to successfully vector the pathogen. To be a successful infection court, the wound must be less than a few days old. This additional requirement in the disease cycle is another reason why losses to oak wilt have been relatively limited.

There is another mechanism of spread for *C. fagacearum*, termed local or underground spread. This mechanism is by means of grafted roots (Fig. 8). Root grafts form when the roots of one tree fuse to those of an adjacent tree of the same or closely-related species. Since *C. fagacearum* is a vascular parasite, functional root grafts provide an effective avenue for spread of the fungus from a diseased to a healthy tree.

The dominance of live oak in central Texas woodlands and its popularity as a shade tree changed the formula for assessing the epidemic potential of *C. fagacearum*. In addition to making acorns, live oak has the ability to reproduce clonally by the formation of root sprouts. This means of vegetative reproduction has several ecological advantages over other tree species when compared to conventional seed production. For example, live oaks have been able to efficiently colonize the former grasslands of central Texas following the control of fires and overgrazing. This ability to rapidly colonize disturbed sites is the reason there are huge monocultures of live oak in central Texas with limited species diversity. These stands are made up of highly interconnected clonal trees with common root systems supplemented by root grafting.

Although vegetative root sprouting may be an efficient means of reproduction, it is particularly detrimental when connected trees are exposed to a vascular parasite such as *C. fagacearum*. Root graft spread is common to oak wilt throughout the range in the U.S., but spread through the live oak common root systems in Texas adds a whole new dimension to oak wilt epidemiology (Fig. 9). Red oaks, such as Spanish oak (*Quercus buckleyii*) and blackjack oak (*Q. marilandica*), play the same role in Texas as they do elsewhere. Fungal mats form on them, but the high heat and dry conditions sometimes diminish the numbers of potential mat-bearing trees. When a contaminated nitidulid introduces *C. fagacearum* into an interconnected stand of live oaks, the pathogen spreads rapidly from tree to tree within the stand. This sort of spread results in the production of very large disease centers (Fig. 10).

The Significance of Live Oaks

The unique response of live oak to oak wilt in Texas has had many implications for our understanding of the disease. Oak wilt went undiscovered in Texas, perhaps at least for 40 years, after the disease was initially described in Wisconsin in 1941. This failure to recognize the disease in Texas probably derived from the unfamiliar symptoms exhibited by live oak. It should also be noted that *C. fagacearum* is a heat sensitive fungus, so the oak forests of the southern U.S. were presumably safe from the disease (Schmidt 1978, Gibbs and French 1980). Although live oak exhibits a high degree of susceptibility to the pathogen in the manner of red oaks, no fungal mats form on live oaks. The ability to reach epidemic proportions in a host population where no external inoculum sources are present for insect transmission reveals the great resilience of the oak wilt pathogen. The ability to adapt to a region with intense high temperature extremes and relatively limited insect transmission clearly shows this pathogen can adapt to new oak forests, regardless of our attempts to predict risk based on our current understanding of the pathogen.

CAN WE CONTROL OAK WILT?

The predominance of live oak as a primary host for *C. fagacearum* also has implications for how we approach the control of the disease in Texas. As with all plant diseases, our ability to control oak wilt is based on our understanding of the biology of the host and the pathogen. Overland spread by nitidulids may be accomplished by avoiding wounding of oaks in the spring, the use of wound paints on fresh wounds, and the cautious movement of firewood. Underground or local spread may be prevented by trenching to break up root grafts and common root systems. Roguing of trees, in order to further damage existing root systems in hopes of destroying pathogen habitat, is also recommended. Intravascular injection of high risk trees with fungicides does not successfully prevent a tree from becoming infected, but it has been shown to be effective in protecting high risk trees from extensive pathogen colonization. As a result, treated trees survive, often with all or most of their crowns intact. New fungicide products continue to be introduced for injections, but most are based on the originally-tested fungicide propiconazole or some closely-related compound. There are numerous trees, including some oak species that are recommended for replanting once the epidemic has abated. Experience has shown that even live oaks planted in remnant oak wilt centers seem to escape the disease indefinitely. Oak wilt control and the related issues of inoculum sources, infection courts, insect vectors, and potential resistance in the live oak population are addressed in greater detail in several of the presentations in this Symposium.

UNANSWERED QUESTIONS

As measures to manage oak wilt improve, it allows us time to consider some of the broader issues concerning this important disease. One of those issues is the origin of the pathogen and the potential for expansion into valuable, unaffected resources. The analysis of the range of a plant pathogen often reveals clues as to where that pathogen may have originated, but in the case of oak wilt this has not been the case. The current range in Texas reflects some revealing attributes of the disease that also occur in the oak wilt range nationally. Oak wilt in Texas continues to be a problem largely in the central portion of the State, where the tree types are dominated by the oak-juniper woodlands. There has been no encroachment into the east Texas pineywoods, where susceptible oak species occur in large numbers (Fig. 1).

The lack of expansion to the west and south in Texas is understandable due to sparse host type, but the failure of the pathogen to disperse into east Texas remains a mystery. A similar phenomenon occurs throughout the range of oak wilt in the U.S. In several states, the pathogen has failed to encroach into forests where there are susceptible trees and no perceptible climatic limits. If we do not have a good understanding of where *C. fagacearum* might have come from, then our ability to predict the impact it may continue to have elsewhere may also be flawed. This issue is particularly timely due to the increasing concern for regulating and assessing the risks of exotic, invasive species. Other lines of evidence on the origins of *C. fagacearum* are discussed by authors elsewhere in this Symposium.

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Table 1. Results of ground truthing randomly selected polygons at Fort Hood, TX.

Cause	No. Centers	% Total
Oak Wilt	82	69
Military Ops	1	0.8
Unknown	8	6.7
Brush Piles	23	19.3
Blow down	1	0.8
Fire	4	3.3

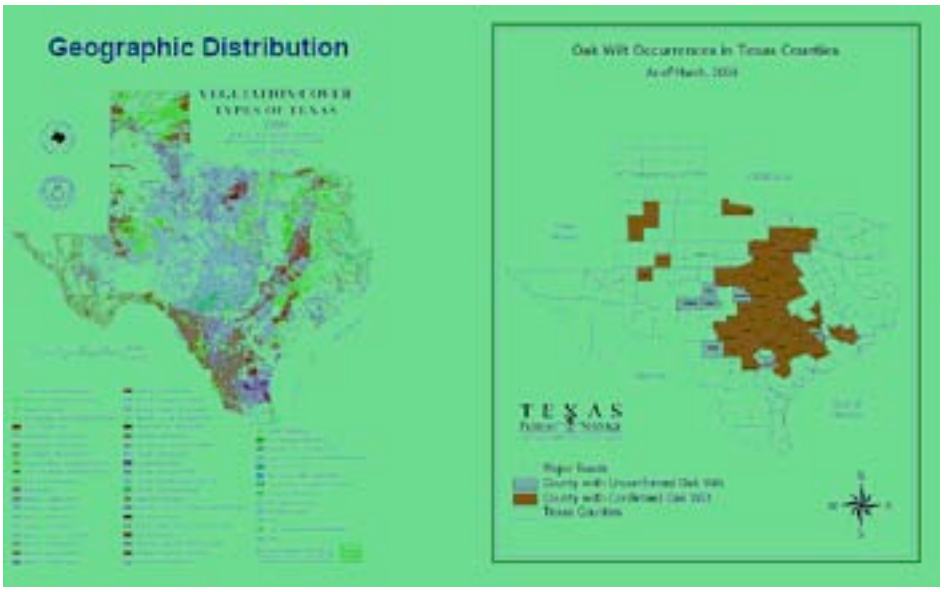

Figure 1. Vegetative cover types and range of oak wilt in Texas.

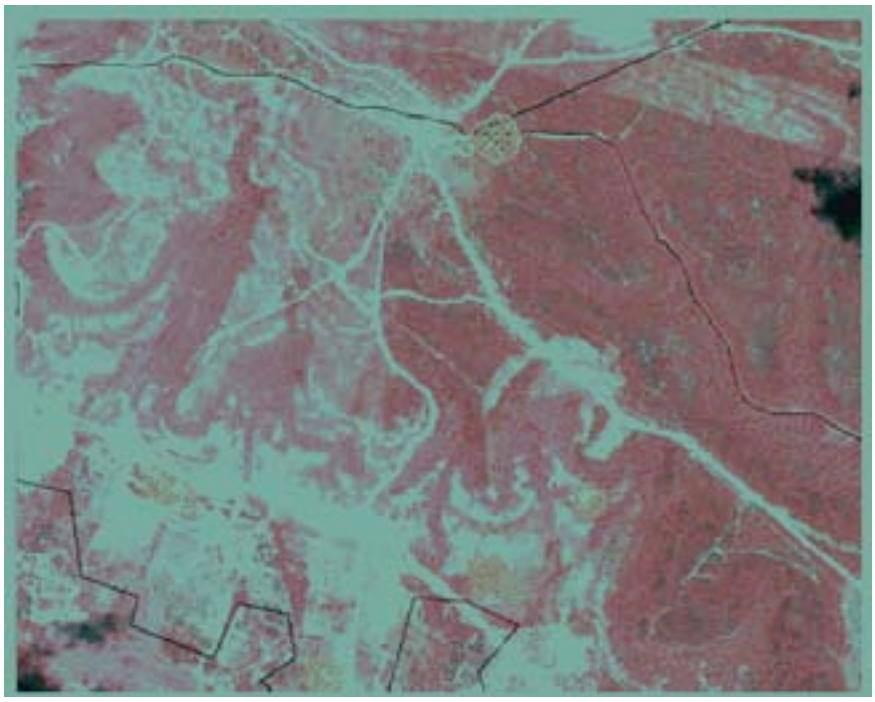

Figure 2. Example of the IKONOS 1-meter satellite imagery used to survey for oak wilt at Fort Hood, TX. Yellow polygons are areas identified by the photointerpreter as oak mortality.

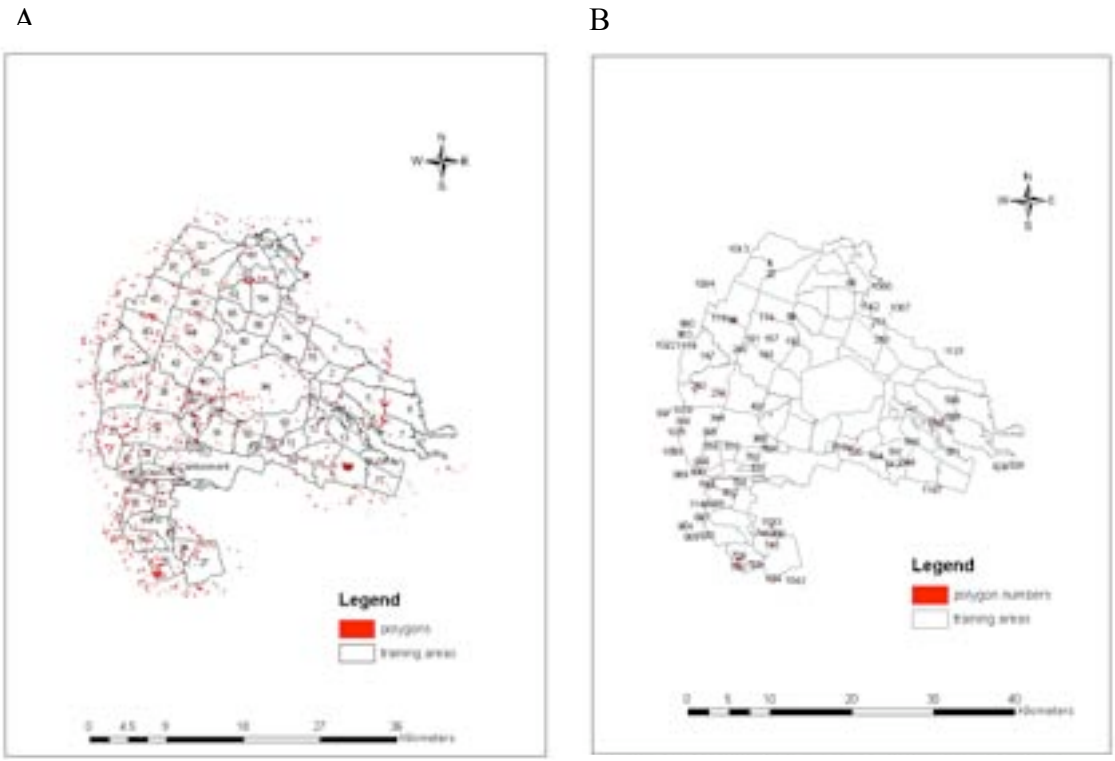

Figure 3. Map of Fort Hood, TX, with training areas and the locations of the 1164 polygons interpreted as oak mortality (A) and the 119 randomly selected polygons selected for ground truthing (B).

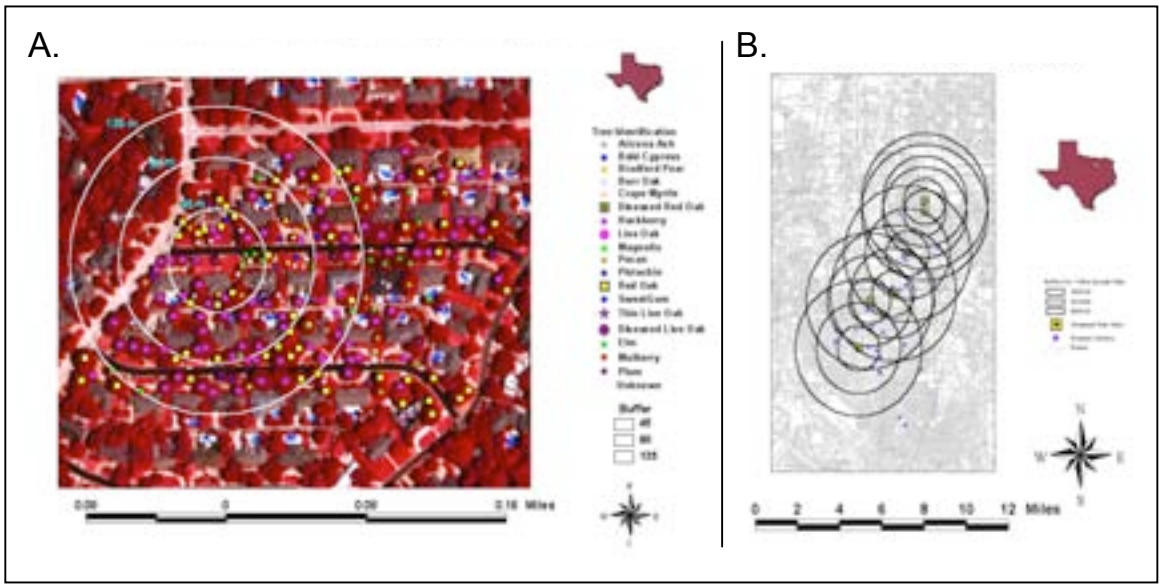

Figure 4. Illustration of the risk of annual spread of *C. fagacearum* locally through root connections (A) and by overland by insect vectors (B) in Dallas, TX.

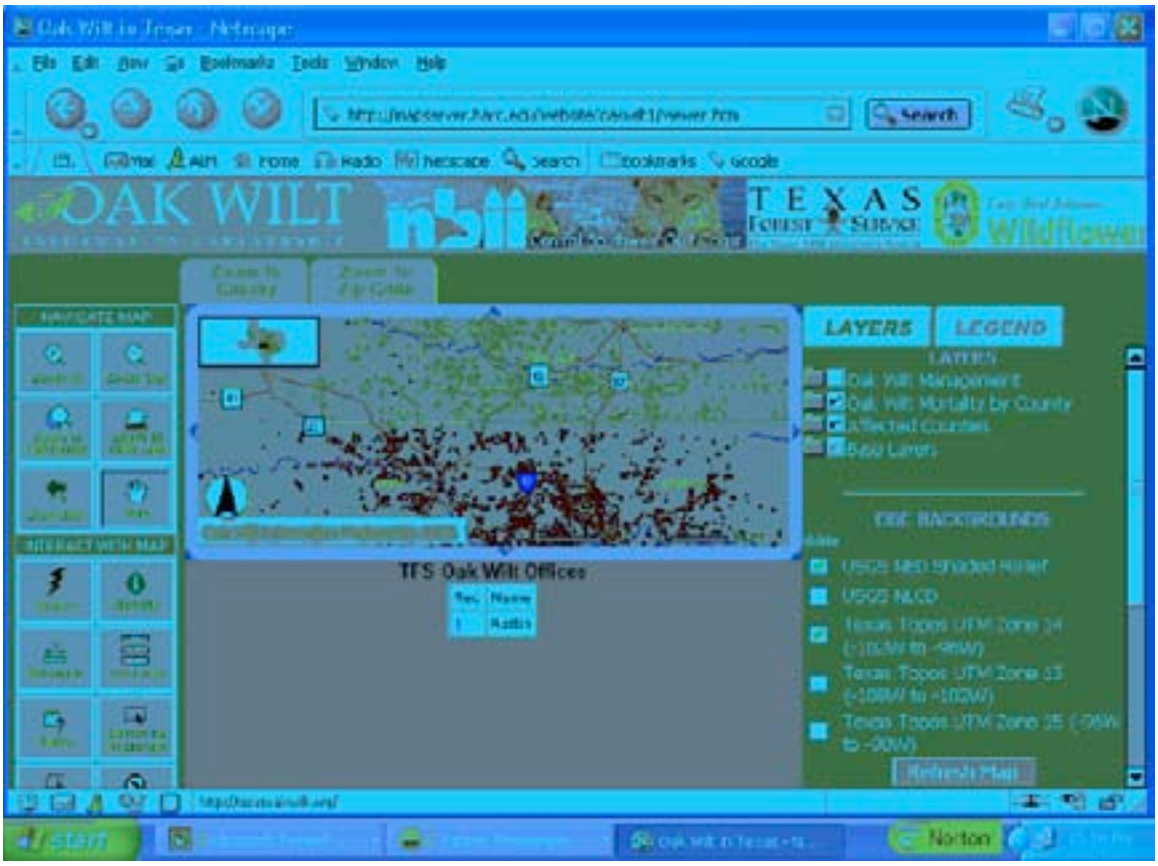

Figure 5. Interactive website (www.texasoakwilt.org) illustrating the incidence of oak wilt in Kerr/Kendall counties, TX. Red polygons are areas of oak mortality identified by Texas Forest Service foresters during aerial surveys.

Figure 6. Dead red oaks near Lampasas, TX, and typical red oak foliar symptoms (insert).

Figure 7. Dying live oaks near Lampasas, TX, and typical live oak foliar symptoms of infection (inserts).

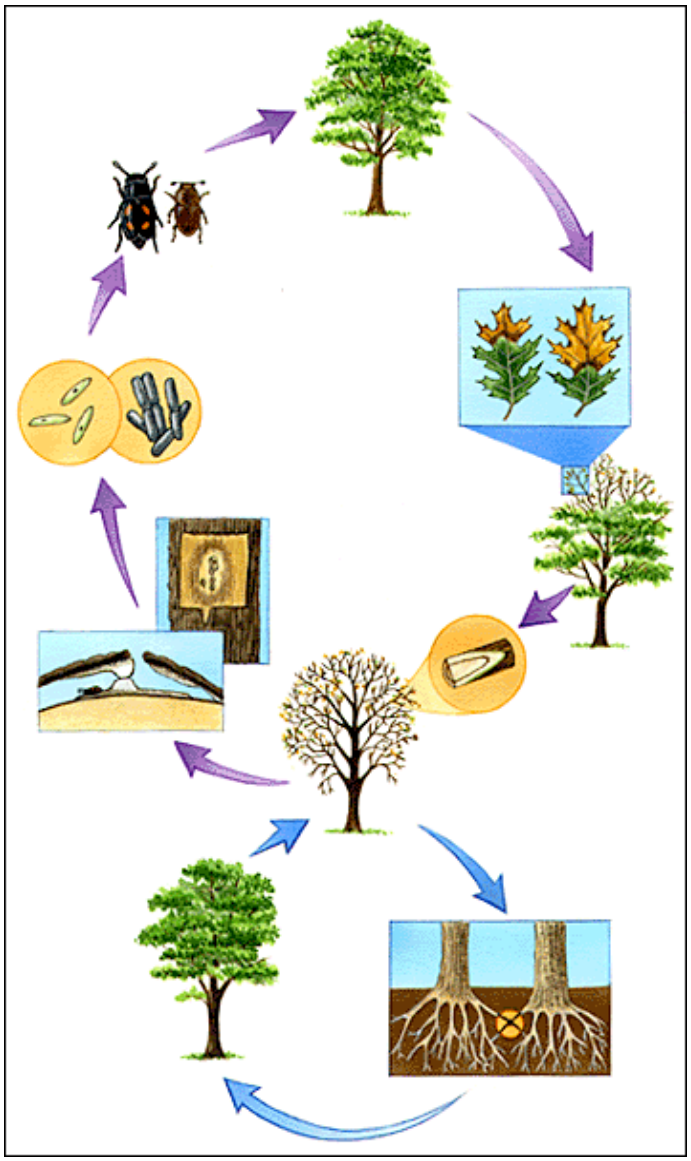

Figure 8. Oak wilt disease cycle (O'Brien et al. 2000).

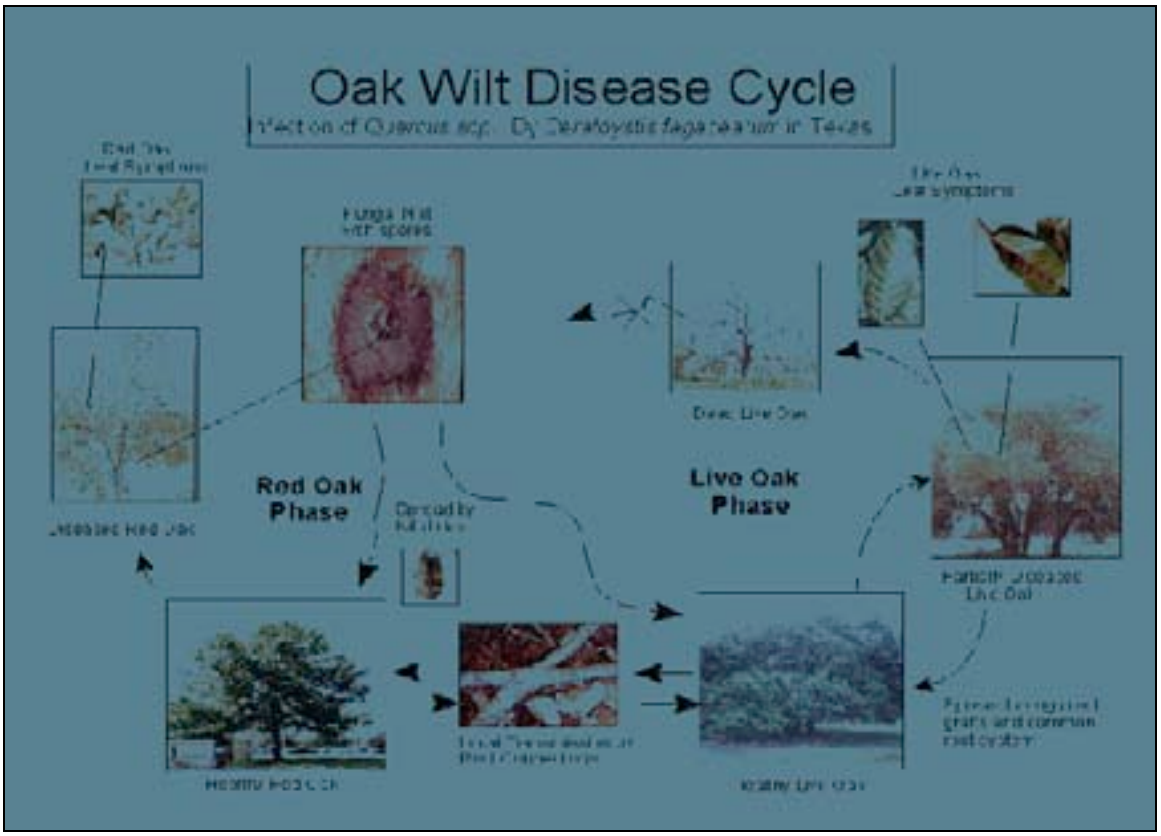

Figure 9. Oak wilt cycle in Texas, with emphasis on the live oak phase of the disease.


Figure 10. Remnants of a large, old oak wilt center near Lampasas, TX.

EPIDEMIOLOGY AND OCCURRENCE OF OAK WILT IN MIDWESTERN, MIDDLE, AND SOUTH ATLANTIC STATES

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ABSTRACT

In Midwestern, Middle, and South Atlantic states, the oak wilt fungus (*Ceratocystis fagacearum*) is transmitted from diseased to healthy oaks below ground via root grafts and above ground via insect vectors. Recent studies have identified insect species in the family Nitidulidae that likely account for the majority of above-ground transmission during spring in several Midwestern states based on frequencies of fungus-contaminated beetles dispersing in oak stands and visiting fresh wounds. Other investigations have utilized quantitative and spatial data to predict root-graft spread in red oak stands. Although the disease is widely distributed in the regions, disease severity ranges from low to high among the regions and within states of the Midwestern region. Knowledge of spread frequencies and relationships between disease spread/severity and various physiographic factors is important in the development of tools for effective disease management.

Key words: *Ceratocystis fagacearum*, disease spread, Nitidulidae

New oak wilt infection centers (= foci) are the result of above-ground transmission of the pathogen (*Ceratocystis fagacearum* (Bretz) Hunt) by animal vectors, primarily insects. Outward expansion of foci from the initial infection(s) occur below ground when fungal propagules move through vascular root connections between a diseased and a nearby healthy oak. A basic understanding of these general means of oak wilt spread was developed at least 40 years ago and was a result of intense research activity that occurred in the 20 years following the recognition and description of *C. fagacearum* as the causal organism (Henry et al. 1944). An excellent review of oak wilt transmission was published in 1980 (Gibbs and French 1980). Modifications of this understanding were subsequently made in response to the first recognition of oak wilt in Texas and the specifics of pathogen spread that were elucidated thereafter (Appel 1995). Refinements and additional details of spread in the Midwestern States, Middle, and South Atlantic states have also occurred during the past three decades.

Documentation of disease foci occurrence across landscapes is available in the older literature, but factors influencing the distribution of *C. fagacearum* are complex and have been poorly understood (MacDonald 1995). Landscape-level epidemiological models and emerging spatial tools are providing increased understanding of various factors and features correlated with disease patterns across landscapes (e.g., Bowen and Merrill 1982, Menges and Loucks 1984, Appel and Camilli 2005). Quantitative data available in published literature can be valuable in such investigations; disease prediction efforts may also benefit from such information. New or refined tools for predicting, preventing, monitoring, or managing spread of the oak wilt fungus in forest landscapes arise from quantitative and spatial studies of the disease occurrence and spread.

The epidemiology of oak wilt outside Texas was most recently reviewed by Prey and Kuntz (1995). In this paper, aspects of the epidemiology and occurrence of oak wilt within and among

states of the Midwestern, Middle Atlantic, and South Atlantic regions of the U.S. are considered. Particular attention is paid to results of recent studies (< 30 years). The frequencies of above- and below-ground spread occurrence, factors influencing spread, and relationship of one spread type to the other are discussed within the context of disease incidence and severity observed in these landscapes. Lastly, applications of this knowledge for effective oak wilt management in these regions are also briefly discussed.

EPIDEMIOLOGY AND DISEASE OCCURRENCE

In the Midwestern and Middle and South Atlantic States, *Ceratocystis fagacearum* is transmitted from diseased to healthy oaks either above ground by insects or below ground through functional root grafts. The pathogen's spread through root grafts results in the progressive enlargement of existing oak wilt foci, whereas insect vectors are responsible for the introduction of the pathogen to previously-unaffected forest patches or non-systematically to healthy trees within contiguous forests where the disease is already established.

Initiation of New Disease Foci

Squirrels, birds, and several insect families have been implicated as above-ground vectors of the pathogen, but little published data exists to support these assertions for the first two groups. Overall, insects are considered to be responsible for the vast majority of above-ground spread. Sap beetles (Coleoptera: Nitidulidae) and oak bark beetles (Coleoptera: Curculionidae: Scolytinae) are commonly cited as the main vector groups while ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), certain buprestids (Coleoptera: Buprestidae) and cerambycids (Coleoptera: Cerambycidae) may be occasional vectors (Merrill and French 1995). Aspects of the two main vector groups are presented here.

Sap Beetles. The sequential conditions needed for successful transmission by sap beetles include: a) the availability of viable inoculum (i.e., oak wilt fungal mats), b) inoculum acquisition by vector species, c) dispersal of contaminated insects, d) attraction of pathogen-contaminated insects to fresh, xylem-penetrating wounds on healthy oaks, and e) receptivity of fresh wounds to infection. The factors influencing several of these conditions have been previously reviewed (Gibbs and French 1980). In general, the highest frequency of sap beetle-mediated transmission occurs during spring months when peaks in mat abundance, contaminated insect density, and host susceptibility coincide. The starting and ending dates of the critical spring period (i.e., for sap beetle transmission) do change with increasing latitude. For example, the month with the highest risk of transmission in central Missouri is April, but in Minnesota it is May based on frequencies of pathogen-contaminated sap beetles captured in fresh wounds on healthy oaks in each state (Juzwik, Skalbeck and Neuman 2004, Hayslett, Juzwik and Moltzan 2008).

Very low to no transmission occurs in November through February when no or only greatly deteriorated mats are present, vector species are in over-wintering locations, and pathogen infection of wounds rarely if ever occurs due to low ambient temperatures. Although late-summer and fall mat production is common and often comparable to levels during spring in Minnesota (Juzwik 1983), the contaminated insect densities of two dispersing sap beetle vector species were low between August and mid-October (Ambourn, Juzwik and Moon 2005) and fresh wounds were not attractive to such pathogen-contaminated species during this same time period (Juzwik et al. 2006). Climatic conditions affect the onset, duration, and abundance of mat

production during spring in Minnesota (Juzwik 1983). Average minimum February temperature and total spring precipitation were correlated with number of mats per tree based on mixed effects modeling results (McRoberts and Holdaway, unpublished report on file with USDA Forest Service, St. Paul, MN).

Oak species composition and species densities in oak forests affects the frequency and abundance of inoculum available for vector spread and, theoretically, the frequencies of new center establishment in the landscape. In general, oak wilt fungal mats are commonly formed on red oak species and less frequently to rarely on white oak species. Mats have been commonly observed on the predominant red oak species in the three regions. Contrary to earlier reports (e.g., Berry and Bretz 1966), mats have been found to commonly occur on recently wilted red oaks in Missouri within the past decade (Juzwik and Moltzan, personal observation) and sap beetles commonly inhabit the mats. Because of their propensity for inoculum production, red oaks are a significant factor in *C. fagacearum* spread and disease intensification in forest landscapes of the three regions.

Mat formation has also been reported on inoculated bur oak (*Q. macrocarpa*), a white oak species, in Iowa and Wisconsin (Engelhard 1955, Nair and Kuntz 1963) and, anecdotally, on bur oak in natural landscapes in Minnesota. Furthermore, *C. fagacearum* infected bur oaks may produce new mats in successive years on trees with recurrent wilt symptoms (Nair 1964). Pathogen contaminated sap beetles were associated with the small sporulating mats found on this species (Nair and Kuntz 1963). Mats apparently infrequently to rarely occur on *Q. alba*, another white oak species (Cones 1967). Thus, bur and white oak would appear to play a role, albeit likely minor, in the overland spread of *C. fagacearum* in the three regions.

Oak Bark Beetles. Sequential events leading to successful transmission by oak bark beetles (*Pseudopityophthorus minutissimus* and *P. pruinosus*) include: a) reproductive colonization of recently-wilted oaks, b) acquisition of viable pathogen propagules by teneral adults prior to emergence from colonized, diseased oaks, c) dispersal of contaminated insects, and d) inoculation of healthy oaks during maturation feeding in the crowns of healthy oaks (Ambourn, Juzwik and Eggers 2006). Wounds created by the beetles during feeding are considered suitable infection courts for the pathogen. The frequencies of reproductive colonization by *Pseudopityopthorus* spp. in oaks and dispersion of pathogen-contaminated beetles apparently differ greatly among, and even within parts of, the Midwestern, Middle Atlantic, and South Atlantic states (True et al. 1960, Berry and Bretz 1966, Rexrode 1969, Ambourn, Juzwik and Eggers 2006). The highest frequency of oak bark beetle transmission likely occurs during mid- to late spring. The frequencies of pathogen-contaminated *P. minutissimus* dispersing in oak wilt centers in east central Minnesota were 4 to 13 per thousand in May and June (Ambourn, Juzwik and Eggars 2006).

Predominant Insect Vectors. Historically, sap beetles have been considered the primary vectors in six of the seven states in the Midwestern region. Oak bark beetles were cited as the main vectors in Missouri (Rexrode and Jones 1970); however, recent evidence supports the importance of sap beetles in transmission in the state (Hayslett, Juzwik and Moltzan 2008). Sap beetles as well as oak bark beetles and ambrosia beetles have all been considered vectors in the Middle and South Atlantic states (Merrill and French 1995). Sap beetle species are more efficient vectors than oak bark beetles in Minnesota (Ambourn, Juzwik and Eggers 2006).

In separate Illinois and Minnesota studies, Menges and Loucks (1984) and Shelstad et al. (1991) found higher efficiencies of vector spread over short distances and that longer distance spread occurrences are highly stochastic. In Minnesota, contaminated insect densities of the dispersing sap beetle *Colopterus truncatus* were higher when populations were sampled in active disease centers compared to those in oak wilt–free stands (Ambourn, Juzwik and Moon 2005).

Rates of New Foci Occurrence. Landscape-level estimates of the frequencies of new oak wilt centers established via overland spread are available for four Midwestern states. Rates range from a high of 0.42 new centers/ha/yr for Minnesota and Wisconsin (Anderson and Anderson 1963), to much lower in Missouri (< 0.07 new foci/ha/yr) (Jones and Bretz 1958), and lowest in Illinois (≤ 0.006 new foci/ha/yr) (Menges 1978). In comparison, the frequencies of new center occurrence in Pennsylvania and West Virginia are one-tenth to one-hundredth of the lowest rates for the Midwestern states (Merrill 1967). The rates for North Carolina and Tennessee were even lower (< 0.0006 new foci/ha/yr) than for Pennsylvania and West Virginia (Boyce 1959).

Expansion of Disease Foci

Root Grafting and Pathogen Spread via Grafts. Root grafts are known to occur in numerous oak species (Graham and Bormann 1966). When the roots of trees in close proximity graft together and form a functional union, the biological processes of one tree are strongly influenced by those of the connected tree(s) (Epstein 1978).

Self-, intra-specific-, and inter-specific root grafting occur in oaks. Self-grafting is common in red oaks and may facilitate movement of the fungus among the major roots without first passing through the root collar. In a Minnesota study, *C. fagacearum* was isolated from 14 of 62 self grafts assayed from 12 diseased northern pin oaks (Blaedow and Juzwik 2007). Frequencies of intra-specific grafting occurrence vary by species, site, and geographic region. The highest frequencies (over 70%) of such grafting have been reported for *Q. ellipsoidalis* and the lowest for *Q. macrocarpa* (6%), both in central Wisconsin (Parmeter, Kuntz and Riker 1956). Frequencies of inter-specific grafting are generally lower than intra-specific. Such grafting has been reported between species within the red oak group and between species of the red and white oak groups. The highest inter-specific grafting frequencies (43%) reported in the literature occurred between *Q. velutina* and *Q. alba* in North Carolina (Boyce 1959). Grafting between *Q. macrocarpa* and *Q. ellisoidalis* is not uncommon in Minnesota (Juzwik, personal observation).

Other factors influencing frequencies of root grafting, and hence of *C. fagacearum* spread, are basal area (combined measure of tree density and tree diameters), soil depth, soil texture, and occurrence of non-oak species. The percent oak mortality attributable to root-graft transmission and average disease center size increase with increasing percent red oak composition in Midwestern forests (Menges and Loucks 1984). Root grafting frequency was higher in shallower and/or restricted soils in West Virginia (e.g., True and Gillespie 1961, Gillespie and True 1959). Frequencies of root graft spread also increase from heavier textured soils (silt loam) to light textured soils (e.g., sands) (Menges 1978, Prey and Kuntz 1995). The occurrence of non-oak species in affected stands can either reduce the incidence of root graft spread when inter-mixed among the oaks or stop the below-ground spread when the type changes within the stand.

Rates of Disease Foci Expansion. In Midwestern states, the average radial expansion of oak wilt foci ranges from 1.9 to 7.6 m/yr with the highest rates occurring on deep sand soils of the Anoka Sand Plains, Minnesota, and up to 12 m/yr on sandy soils in the Upper Peninsula of Michigan (Bruhn and Heyd 1991). The oak wilt-associated mortality in the latter type sites average 8 to 11 red oaks/ha/yr. Bur oaks die at a much lower rate (≤ 1/ha/yr) (French and Bergdahl 1973). Radial expansion rates in distance measurements for the Middle and Atlantic states are not reported in the published literature. However, mortality rates attributed to root-graft spread are available. In Pennsylvania, mortality rates varied from 1 to 3 oaks/center/yr (Jefferey and Tressler 1969, Jones 1971) while the lowest rates, 0.19 to 0.39 oaks/center/yr, have been reported for West Virginia (Jones 1971, Mielke, Hayes and Rexrode 1983).

Frequencies of Spread Type in Relation to Disease Occurrence

Low frequency to rare occurrence of *Ceratocystis fagacearum* spread across larger distances (e.g, > 300 m), especially from an affected forest patch to a disjunct, wilt-free patch obviously constrains the frequency of within patch spread and development of oak wilt across the landscape (Fig. 1, as modified from Menges and Loucks 1984). If the long-distance event frequency were to increase (e.g., through increased transport frequency of inoculum-laden logs to an area), then the frequencies of within-patch vector spread and root-graft transmission would correspondingly increase, especially if species composition and density, landform, and soils were conducive to spread. Similarly, the frequency of within-patch vector spread which is largely dependent on red oak species abundance directly affects the incidence of new foci within a forest patch and the opportunity for increased root-graft spread, such as occurs in several Midwestern states (Fig. 1A). However, when root-graft frequency is lower, the temporal and numeric incidence of mat-bearing oaks should logically be lower and the probability for both within and among patch vector spread correspondingly lowered as occurs in the Middle and South Atlantic states and southern portions of the Midwestern states (Fig. 1B).

A high red oak component is critical to sustaining an epidemic and thus of increasing the spread frequency. Using the ratio of red oak to white oak volume for a state's oak forest resources (derived from Smith et al. 2004), the calculated ratios for states sustaining oak wilt epidemics ranged from 1.32 to 2.58 for Minnesota, Michigan, and Wisconsin. In contrast, the ratios for Middle and South Atlantic states are < 1.0, except for Pennsylvania with a ratio of 1.27 and < 1.0 in Iowa and Missouri in the Midwest region.

Oak Wilt Occurrence and Other Factors

Topographic position is correlated with oak wilt occurrence. Furthermore, landform and soils are important factors in explaining oak wilt incidence and severity. In areas of Pennsylvania, Wisconsin, and West Virginia with obvious topographic relief, oak wilt was reported to be common on upper slopes and ridge tops (Anderson and Anderson 1963, Cones and True 1967, Bowen and Merrill 1982). Oak wilt is also very common in areas of low topographic relief in southeastern Iowa and different parts of Michigan, Minnesota, and Wisconsin.

In general, oak wilt is most severe on dry and dry-mesic sites that are also considered to be low in productivity in the three regions addressed here. Oaks are particularly well-suited for successfully invading disturbed sites characterized by these moisture regimes and compete particularly well for stand space and canopy position against other woody species on dry sites (Johnson, Shifley and Rogers 2003). Such sites are also described as being "poor quality" or characterized by low site index. For oaks, in general, poor sites have site indices of < 50,

medium sites have indices between 50 and 65 while good sites are defined by indices of > 65. A much higher percentage (53%) of oak wilt foci occurred on poor sites in Hampshire Co., WV, than would be expected if randomly distributed (Cones and True 1967). In mixed oak stands of the Sinnissippi Forest, Illinois, oak wilt mortality (vector and root-graft attributed) was significantly higher on poor sites than on medium or good sites for mixed oak and all composition of oak forests (Menges 1978).

Conclusions

Although oak wilt is widely distributed in the Midwestern, Middle and South Atlantic states (http://www.na.fs.fed.us/fhp/ow/maps/ow_dist_fs.shtm, last accessed May 17, 2007), disease severity when measured by numbers of oaks killed per hectare, area affected by oak wilt, and/or number of disease foci per hectare differs greatly between the regions and within the Midwestern states. For example, oak wilt has been reported in all but 3 of 55 counties in West Virginia, disease incidence is sporadic and severity (i.e., oak mortality per disease center) is low. In contrast, oak wilt has been reported in only 25 of over 65 counties with significant amounts of oak forests in Minnesota.

In general, disease severity is very high on the deep sand soils north of Minneapolis and St. Paul and east of Rochester, Minnesota (Albers 2001). In Missouri, oak wilt is widely distributed throughout the Ozarks and central Missouri, although relatively few trees succumb to the disease in each disease center. However, oak wilt severity is more obvious in urban and community forests of the St. Louis and Kansas City metropolitan areas compared to the Ozarks (Bruce Moltzan, personal communication, Missouri Department of Conservation, May 21, 2007). Differences in oak wilt severity can often be explained by physiographic factors and features such as those previously discussed.

IMPLICATIONS FOR DISEASE MANAGEMENT

Knowledge of spread frequencies and significant factors influencing local and higher spatial level spread can, and have been, used to develop tools and strategies to manage oak wilt. Several examples of how such knowledge has been applied to oak wilt management at different spatial scales are discussed by spread type.

Managing Above-ground Spread

The frequencies of short- versus long-distance transmission of *C. fagacearum* by insects support an emphasis on removal of potential spore mat-bearing trees at the site and local control level. Effective use of mechanical disruption to stop transmission via root grafts can be negated when measures to prevent insect transmission are not incorporated, i.e., insect vectors may move the fungus over barrier lines. The impact of infrequent, longer distance (> 300 m) transmission events by insects at the landscape level is also significant in terms of oak loss (Shelstad et al. 1991). Human-transport-mediated, insect transmission of the pathogen through intrastate and interstate movement of firewood and logs from recently-felled, diseased trees is an infrequent event but one that can also lead to significant future losses. Such knowledge is considered when natural resource managers develop strategies to prevent the expansion of the oak wilt range in their states and beyond.

Knowledge of the frequencies of pathogen-contaminated beetles visiting wounds and dispersing in oak stands was utilized in conducting an analysis of the relative probability of disease spread into unaffected oak forests undergoing timber stand improvement or harvest

activities (Juzwik, Cummings-Carlson and Scanlon 2008). Statewide guidelines for preventing or reducing oak wilt spread during harvesting activities in oak timberland were based on the risk analysis (http://www.dnr.state.wi.us/org/land/forestry/fh/oakWilt/guidelines.asp, last accessed May 20, 2007).

Managing Below-ground Spread

Statistical analyses and modeling efforts defined the relationship between oak tree diameter and inter-tree distance to the probability of below-ground pathogen spread in Wisconsin (Menges 1978, Menges and Loucks 1984). Later research built on this knowledge developed a model to describe the probability of root graft transmission within one year at high confidence levels based on diameters of source and target trees and the distances between them on loamy sand and sand soils (Bruhn et al. 1991). This model was then used to generate a table to guide foresters in root-graft barrier line placement in Wisconsin (Carlson and Martin 2005).

Predicting General Disease Spread and Severity

Recent efforts to develop risk maps for major insect and disease organisms threatening U.S. forests included an analysis of the potential for oak wilt to cause significant oak mortality within a 15-year period (http://www.fs.fed.us/foresthealth/technology/nidrm.shtml, last accessed May 20, 2007). Both scientific- and experienced-based knowledge were used to develop criteria to rank oak forest susceptibility and/or vulnerability to oak wilt occurrence and severity. The criteria that were variously weighted included proximity to existing oak wilt foci, change in human population, road density, oak stocking level, average number of annual storm events, and stand size.

SUMMARY

Oak wilt continues to cause significant losses of oaks in Midwestern oak forests. The disease is widely distributed in the Midwestern, Middle, and South Atlantic states, but disease severity ranges from low to high among the regions and within states of the Midwestern region. Documentation of spread frequencies and elucidation of factors and physiographic features correlated with low to high disease severity in the landscape have proven useful in developing tools or strategies to prevent, detect, and manage the disease in rural and community forests.

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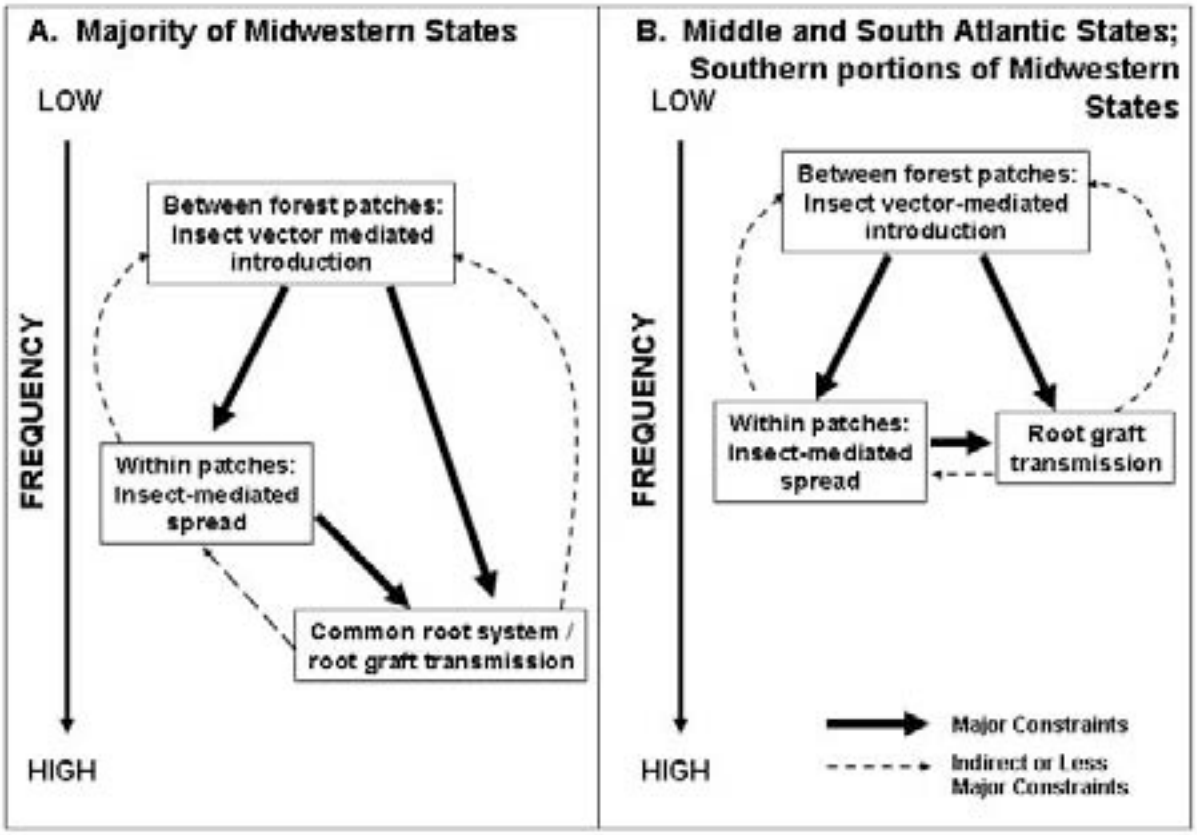

Figure 1. Models of oak wilt occurrence and spread in Midwestern, Middle Atlantic, and South Atlantic states. Figure 1A reproduced with permission from Menges and Loucks, 1984; Figure 1B is a modification of 1A by J. Juzwik.

USING CLASSIFICATION TREE ANALYSIS TO PREDICT OAK WILT DISTRIBUTION IN MINNESOTA AND TEXAS

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ABSTRACT

We developed a methodology and compared results for predicting the potential distribution of *Ceratocystis fagacearum* (causal agent of oak wilt), in both Anoka County, MN, and Fort Hood, TX. The Potential Distribution of Oak Wilt (PDOW) utilizes a binary classification tree statistical technique that incorporates: geographical information systems (GIS); field sample data; commonly available, inexpensive, coarse-resolution auxiliary data; and satellite imagery from both Landsat Thematic Mapper (TM) and SPOT to predict the spatial distribution of oak wilt. Two types of model evaluations were conducted - a ten-fold cross validation and an assessment using additional oak wilt data that had been verified in the field. These evaluations indicated that at the landscape scale PDOW correctly models the presence of oak wilt, and accurately predicts oak wilt distribution in Anoka County, MN and Fort Hood, TX. Variables that were common for predicting oak wilt distribution in both Anoka County and Fort Hood were: Landsat TM Bands 3, 5, and 7; sand; aspect; and elevation. Additional variables important in Fort Hood included: Spot band 1, stream density, and slope. Variables that were unique and important for Anoka County included: TM Band 4, organic matter, silt, drainage, population, and population change.

Key words: *Ceratocystis fagacearum,* classification tree, decision tree, Landsat TM, spatial statistics, SPOT satellite imagery.

The oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, kills thousands of oak trees (*Quercus* spp.) annually within the US disease range, i.e. 22 eastern states and Texas (Appel and Maggio 1984, Juzwik 2000, O'Brien et al. 2003). Continued spread of the pathogen results in expanding disease foci and establishment of new foci in forests, woodlots, and home landscapes. In Minnesota, oak wilt occurs within 20 counties. Between the years of 1991 - 2001, 6,976 acres were treated of a total estimated 15,359 acres affected (MNDNR 2001). The disease is most severe on deep sand soils in east-central Minnesota. In a one year example, the Minnesota Department of Natural Resources (MNDNR) identified and treated 3,182 acres of infected oak wilt trees in 1998 within Anoka County. The MNDNR projected that, at that infection rate, there would be a two-fold increase in oak wilt by 2008 (MNDNR 2000).

In Texas, in 2007, oak wilt occurred within 60 counties and was estimated to affect a minimum of 6,500 acres (Texas Forest Service 2007). The disease is particularly severe on the Edwards Plateau of central Texas. Within this region, including the Fort Hood military installation, the live oak / Ashe juniper community type is critical habitat for two rare bird species (the golden cheeked warbler and the black-capped vireo) indigenous to the region (Diamond 1997). Oak wilt is a potential threat to live oak in this critical habitat.

Aerial and ground surveys are regularly conducted in both states to detect new disease centers and estimate area of land affected. The recent development of new geospatial techniques and geo-statistical analysis tools offer new methods for displaying oak wilt distribution, predicting disease occurrence in areas where data is lacking, and obtaining estimates of both land area affected and forest areas at risk to the disease.

Statistical techniques such as decision tree models are useful for classification problems where mixes of both continuous and categorical data are available for geospatial analysis. Classification or decision trees are non-linear tests made up of a collection of rules displayed in the form of a binary tree. The rules are determined by a recursive partitioning procedure (MathSoft 1999). Advantages of using decision trees include the non-parametric nature of the

model, ease of interpretation, and the robustness of the test (De'Ath and Fabricius 2000). Classification trees offer a way to describe the spatial continuity that is an essential feature of many natural phenomena (Isaaks and Srivastasa 1989), and have been used to: classify remote sensing imagery (Friedl and Brody 1997, Michaelson et al. 1994, Joy, Reich and Reynolds 2003), predict spatial patterns and develop indicators of hemlock woolly adelgid infestation, (Koch 2005), model *Phythophthora ramorum* (sudden oak death) distribution in California (Kelly and Meentemeyer 2002), model the presence and absence of lichen and past fires in Jalisco, Mexico (Reich, Aguiree-Bravo and Bravo et. al. 2005), and to estimate fuel loads in the Black Hills, SD (Reich, Lundquist and Bravo 2004).

The objective of our study was to determine the feasibility of using a decision tree with commonly acquired spatial datasets and location data collected in Anoka County and at Fort Hood to develop spatially-explicit maps that predict the distribution of oak wilt in these locations. Our analysis utilized field data, remotely sensed satellite data, as well as other spatial data within a geographical information system (GIS). The resulting models were evaluated for their accuracies in predicting presence or absence of the oak wilt disease.

MATERIALS AND METHODS

Study Areas

Anoka County is 110,000 hectares located in east-central Minnesota and occurs largely within the Anoka Sand Plains ecological subsection (MNDNR 1999). The terrain is a broad, flat, sandy plain with gently rolling topography. Soils are largely well-drained fine sands. The vegetation included species associated with oak openings and oak barrens. The predominant oak species are northern pin oak (*Q. ellipsoidalis*), northern red oak (*Q. rubra*) and bur oak (*Q. macrocarpa*).

Fort Hood is approximately 87,900 hectares in size, (Ribanszky and Zhang 1992). Fort Hood is located in Bell and Coryell counties, TX, within the Crosstimbers and Southern Tallgrass Prairie and the northeastern edge of the Edwards Plateau Ecoregions. Vegetation in the area consists mainly of open grasslands or savannah with individuals or mottes of oak (*Quercus* spp); ashe juniper (*Juniperus ashei*); and mixed forest dominated by oak-juniper (Diamond 1997, The Nature Conservancy 1997).

Location and Spatial Data

Presence/Absence (Dependent Variable). For the Anoka County study area, a Dependent Variable GIS Sample Point Theme was created using the Land Management Information Center (LMIC) oak wilt database as our primary data source (Table 1). Many sample locations were acquired from the 1998 LMIC oak wilt "treated" polygon data. Additional LMIC sample locations, coded as "possible active" oak wilt sites during the 1998 growing season, were randomly selected and visited in July and August, 2002. If evidence suggested the sites actually had active oak wilt infection centers in 1998 then GPS (Garmin E-Trex Legend) coordinate system points were collected for the oak wilt positive tree locations. Healthy oak site locations were also acquired during the 1998 growing season and again in September 2004. Of the 489 sample points collected in Anoka County, 156 were identified as being healthy oak sample points, and 333 were identified as having been active oak wilt sites in 1998.

All polygon centroid locations from the LMIC database and our additional sample point locations were merged to create the final dependent variable GIS Sample Point Theme. Healthy

oak wilt sample point locations were assigned a value equal to 1, and oak wilt sample point locations were assigned a value equal to 2. Polygon centroid location points were acquired to create sample points from the polygon (USDA Forest Service, FHTET 2007a).

All Fort Hood dependent variable plot data for oak wilt and non-oak wilt sites were collected in the field during the growing seasons of 2003 – 2004 (Table 1). A systematic cluster plot sampling design was implemented to attain the dependent variable sample data; the ratio was two healthy plots to one oak wilt plot. The cluster plots were configured such that four 10 m x 10 m secondary sampling units (ssu) composed one 20 m x 20 m primary sampling unit (psu). This sampling design was used to avoid periodicity in the resource and to permit plots to occur at random distances for spatial modeling (Reich, Aguirre-Bravo and Bravo 2005).

Independent Variable Data. Twenty-three auxiliary or independent grid themes were constructed for use as independent variables in the Anoka County analysis (Table 2): fourteen were created from two, multi-temporal Landsat 5 TM data sets (May and September 1998). The other nine variables were: aspect, distance-to-lakes, distance-to-streams, drainage, elevation, landform, road density, slope, and stream density.

Twenty four independent variable grid themes were constructed for the Fort Hood analysis (Table 2). Seven were from Landsat 5 TM, another four were from SPOT 5 satellite imagery. The remaining thirteen variables included: aspect, detritus, elevation, forb percent, land cover, landform, organic matter, road density, slope, sand, silt, clay, and stream density. All variables for each study area were collected, aggregated or re-sampled to a 30 m x 30 m spatial resolution.

Stratification of Anoka County Land Area

The southern section of Anoka County has a higher degree of urban coverage than the northern section of the county. To determine whether spatial correlation exists between oak wilt and urban or natural landscape features, and to ensure that the urban condition in the south was not affecting the results of the model for the non-urban area to the north, the county was stratified into urban and non-urban datasets and two models were created.

Spatial Information Databases

Three spatial information databases were created for the study areas: Anoka – urban; Anoka – non-urban; and Fort Hood. To do this, information was extracted from each of the independent variable data themes at the grid cell location coincident with the sample point (Anoka County), or cluster plot (Fort Hood) locations (USDA Forest Service, FHTET, 2007b).

Classification Tree Analyses: Creation of a Disease Map

The Spatial Information Databases were used for the classification tree analyses to predict the distribution of oak wilt. The output from the classification tree was the input for conditional statements (ESRI CON statements, 2000), which were used to create an oak wilt presence or absence raster grid surface for each study area (Figs. 1A and B). Grid theme cells with values of 1 indicated lower probabilities of oak wilt presence (defined as absence). Grid theme cells with values of 2 indicated higher probabilities of oak wilt presence.

Evaluations

There were two evaluations performed in each study area: 1) the initial evaluation estimated as a sample-based misclassification error rate, and 2) the tenfold cross-validation, (Efron and Tibshirani 1993), calculated in S-PLUS© as part of the classification tree procedure.

The sample based misclassification error evaluation was conducted by intersecting oak wilt points and polygons with each of the final surfaces to determine the rate at which we accurately predicted the presence of oak wilt. For the Anoka County urban model, a total of 164 known oak wilt polygons, with a mean size of 0.76, minimum size of 0.07, and a maximum size of 10.01 acres, were used. In the Anoka County non-urban model, a total of 65 known oak wilt polygons, with a mean size of 1.94, minimum size of 0.14, and a maximum size of 13.16 acres, were used. In Anoka County, the predicted PDOW was quantified for three categories; 50, 75, and 100 percent of the assessment polygon. To quantify the number of polygons successfully predicted with oak wilt, the assessment polygon was intersected with the results from the PDOW surface, and then the area of predicted oak wilt within the assessment polygon was divided by the total area of the assessment polygon. The polygons that were accurately predicted as having oak wilt were totaled within each category (i.e., 50, 75, and 100). The total number of polygons from each category was then divided by the total number of assessment polygons used for an overall estimate of accuracy.

The cross-validation procedure validates the tree sequence by shrinking and/or pruning the tree by portioning the data into a number of subsets, fitting sub-tree sequences to these, and using a subset previously held out to evaluate the sequence. This procedure was used to identify the tree size that minimized the prediction error.

RESULTS

Oak Wilt Distribution: Models and Surfaces

Anoka County. There were thirteen terminal end nodes in the urban model, which accounted for 84 percent of the variability. The independent variables important in predicting the presence or absence of oak wilt in the urban model were: sand, TM band 4 (May 1998), TM band 6 (September 1998), aspect, silt, drainage, elevation, and population (Fig. 2A). The Anoka County non-urban model had twelve terminal end nodes, which accounted for 86 percent of the variability in the model. The independent variables important for predicting the presence or absence of oak wilt in the non-urban model were: TM band 3 (May 1998), population change, organic matter, silt, TM band 3 (September, 1998), TM band 7 (May 1998), landform, and TM band 5 (May 1998), (Fig. 2B). Oak wilt is predicted to be present in 51 and 56 percent of the urban and non-urban forests, respectively, of Anoka County (Table 3; Figs. 2A and 2 B).

Fort Hood. There were sixteen terminal nodes, which accounted for 93 percent of the variability in the Fort Hood model. The variables of importance for predicting the potential of oak wilt were: landcover, TM band 7 (May 16, 2003), SPOT 5 band 1 (July 2003), stream density, aspect, TM bands 3 and 5 (May 2003), slope, sand and elevation (Fig. 2C). Oak wilt is predicted to be present on 41 percent of the forest land at Fort Hood (Table 3; Fig. 2C).

Model Evaluations

The classification tree selected through cross validation for each of the Anoka County urban and non-urban models had misclassification errors of 0.1588 and 0.1445, respectively. The misclassification error rate for Fort Hood was 0.0651. The error matrix (Table 4) showed the following for each model:

* Anoka urban model, 83 percent (n = 47) of non-oak wilt sample points and 84 percent (n = 186) of oak wilt sample points were correctly classified,

* Anoka non-urban model, 88 percent (n = 109) of non-oak wilt sample points and 84 percent (n = 147) of oak wilt sample points were correctly classified,
* Fort Hood model, 97 percent (n = 278) of healthy sample points and 83 percent (n = 106) of oak wilt sample points were correctly classified.

A second evaluation was conducted on the oak wilt predictions for each study area using the additional data collected in the field, including 1999 and 2000 LMIC data for Anoka County, These accuracy assessment points and polygons (= test data) were not part of the dataset used to develop the oak wilt models.

The frequency accuracies for the predicted presence of oak wilt in the assessment polygons in Anoka County depended on the proportion of the area of each polygon considered. Accuracies were highest when 50% of the assessment polygons were predicted to have the disease (93 percent urban model; 88 percent non-urban model) and lowest when 100 percent of the polygon were predicted to have oak wilt (70 percent, urban model; 25 percent, non-urban model). Of 34 points known to have oak wilt in the Texas area, 25 (74 percent) were predicted to have the disease using the Fort Hood model.

DISCUSSION AND CONCLUSIONS

We restricted our analyses to variables that were easily obtained and at a minimum cost. We showed that using a classification tree on commonly-acquired datasets could reliably predict the distribution of oak wilt in Anoka County, MN, and Fort Hood, TX. The classification tree technique identified several independent variables that were useful in predicting the potential distribution of oak wilt in the Minnesota and Texas landscapes. The combinations of variables included higher values of Landsat TM Band 3 combined with low organic matter, higher values of Landsat TM Band 7 where there were more streams, and were either low lying or with flattened slopes.

Remotely-sensed satellite data, combined with location data collected in the field, was useful for identifying the presence and/or absence of oak wilt in each study area. The satellite bands selected by the classification tree were Landsat TM Bands 3, 4, 5, 6, and 7 and SPOT Band 1. Since SPOT Band 1 provides similar spectral information to the TM Bands, and the improved spatial resolution was not required, we believe there is no added benefit to including the SPOT data in the future.

Also, just as a western aspect was identified by Bowen and Merrill (1982) as being important in predicting oak wilt in Pennsylvania, aspect was identified as an important variable in modeling oak wilt in both the Anoka County urban model and the Fort Hood model. Although it is possible that aspect may be representing flat terrain.

Stratification by land use, specifically the urban and non-urban condition, did not indicate that land use was an important variable in modeling oak wilt. Future analyses should include the urban and non-urban condition as an independent variable for predicting the potential for oak wilt.

Land cover was used in the Fort Hood model but not in either of the Anoka models. As one would expect, the most important variable for predicting oak wilt in Fort Hood was for the sample location to occur in deciduous forested land cover types.

Although our potential distribution of oak wilt might be considered a theoretical construct (Felicisimo et al. 2002), our accuracy assessment using additional oak wilt locations establishes that the classification tree analysis of large-scale, commonly-acquired data can be successfully used to construct a model for predicting the potential distribution of oak wilt in both the

Minnesota and Texas landscapes. The authors recommend the continued investigation of such techniques on other forest pest species.

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Table 1. Each sample point dataset containing the presence and absence oak wilt data for each study area: Anoka County urban, Anoka County non-urban, and Fort Hood became the dependent variable in a classification tree against which the independent variables were tested for correlation:

Anoka County Dependent Variable Sample Point Theme
A. Oak wilt presence and absence field sample data from the Land Management Information Center (LMIC), Forest Health, Oak Wilt, treated site polygon data, 1998.
B. Field visits by the USDA FS NCRS Forest Disease Unit and the Forest Health Technology Enterprise Team (FHTET) to the LMIC, Forest Health, “active” oak wilt sites.
C. Field visits by the USDA FS NCRS Forest Disease Unit and FHTET to randomly selected healthy oak forest sites.

Fort Hood Dependent Variable Sample Point Theme
A. Field visits by the USDA FS Forest Health Technology Enterprise Team (FHTET), Region 8, Texas A & M and Texas Department of Forestry personnel, to randomly select “healthy and “active” oak wilt sites.

Table 2. All independent variables used to determine the level of correlation with the dependent variable in the binary classification trees. Anoka County, MN: A-K; Fort Hood, TX: L-Z.

Anoka County Independent Variables (A-K)

A.	Aspect (compass direction), derived from the USGS DEM using ArcView Spatial Analyst (ESRI) aspect function; North = A, Northeast = B, East =C, Southeast = D, South =E, Southwest = F, West = G, Northwest = H.
B.	Distance to Lakes: USGS 1:100,000 DLG data, measured using ArcView Spatial Analyst, distance in meters from feature function.
C.	Distance to Streams: USGS 1:100,000 DLG data, measured using ArcView Spatial Analyst, distance in meters from line feature function.
D.	Drainage: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
E.	Elevation: derived from the USGS 30 *m* (1:24000) DEM.
F.	Landform: (independent of slope), created from a custom ArcView Avenue application, which uses an irregular 3 x 3 kernel, where positive values indicate concavity and negative values indicate convexity, to calculate landform from a USGS DEM. A zero value indicates flat terrain (McNab, 1989).
G.	Seven Bands from Landsat Thematic Mapper Satellite Imagery: Path 27 Row 29 bands 1-7, acquired May 1998.
H.	Seven Bands from Landsat Thematic Mapper Satellite Imagery: Path 27 Row 29 bands 1-7, acquired September 1998.
I.	Road Density: measured using ArcView Spatial Analyst, distance in meters from line feature function. It was calculated as the sum of roads within 400 x 400 meter grid surfaces. Roads include City Streets, County Roads, and TWP Roads from USGS 1:24,000 data and Major and Ramp roads from MN Department of Transportation data.
J.	Slope degrees: derived from the USGS DEM using ArcView Spatial Analyst (ESRI) slope function
K.	Stream Density: from Minnesota Department of Natural Resources, MN Wetlands and Surface Water Resources data set; calculated as the sum of all stream surface area within 400 x 400 meter surface grids.

Fort Hood Independent Variables (L-Z)

L.	Aspect (compass direction), derived from the USGS DEM using ArcView Spatial Analyst (ESRI) aspect function; North = 1, Northeast = 2, East = 3, Southeast = 4, South = 5, Southwest = 6, West = 7, Northwest = 8.
M.	Clay: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
N.	Detritus: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
O.	Elevation derived from the USGS 30 meter resolution DEM (1:24000 scale).
P.	Forb percent: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
Q.	Landcover: Derived from Landsat Imagery collected 5/16/2003; a = open, b = coniferous, c = deciduous.
R.	Landform: (independent of slope), created from a custom ArcView Avenue application, which uses an irregular 3 x 3 kernel, where positive values indicate concavity and negative values indicate convexity, to calculate landform from a USGS DEM. A zero value indicates flat terrain (McNab, 1989).
S.	Seven Bands from Landsat 5 TM Satellite Imagery: Path 27 Row 38, bands 1-7, acquired 5/16/2003.
T.	Organic matter: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov

U. Road Density: The Nature Conservancy, Fort Hood Project, Fort Hood, Texas.
V. Slope degrees: derived from the USGS DEM using ArcView Spatial Analyst (ESRI) slope function.
W. Sand: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
X. Silt: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); http://soildatamart.nrcs.usda.gov
Y. SPOT 5 Satellite: Multi-spectral Imagery: Bands 1-4 acquired 7/29/ 2003
Z. Stream Density: The Nature Conservancy, Fort Hood Project, Fort Hood, Texas; calculated as the sum of all stream surface area within 400 x 400 meter surface grids.

Table 3. Proportion of forest predicted with oak wilt and healthy oak in the urban and non-urban models for the Anoka County and Fort Hood study areas.

	Urban	Non-Urban	Anoka County Totals	Fort Hood Totals
Forested Area	26,179 acres	54,889 acres	81,068 acres	107,665 acres
Oak Wilt	13,452 acres 51 percent	31,014 acres 56 percent	44,466 acres 55 percent	44,192 41 percent
Healthy Oak	12,727 acres 48 percent	23,875 acres 43 percent	36,602 acres 45 percent	63,473 59 percent

Table 4. Misclassification error rates for each study area:

A. Non-Urban	Absent	Present
Classified Absent	96	24
Classified Present	13	123

B. Urban	Absent	Present
Classified Absent	39	29
Classified Present	8	157

A. Fort Hood	Absent	Present
Classified Absent	271	18
Classified Present	7	88

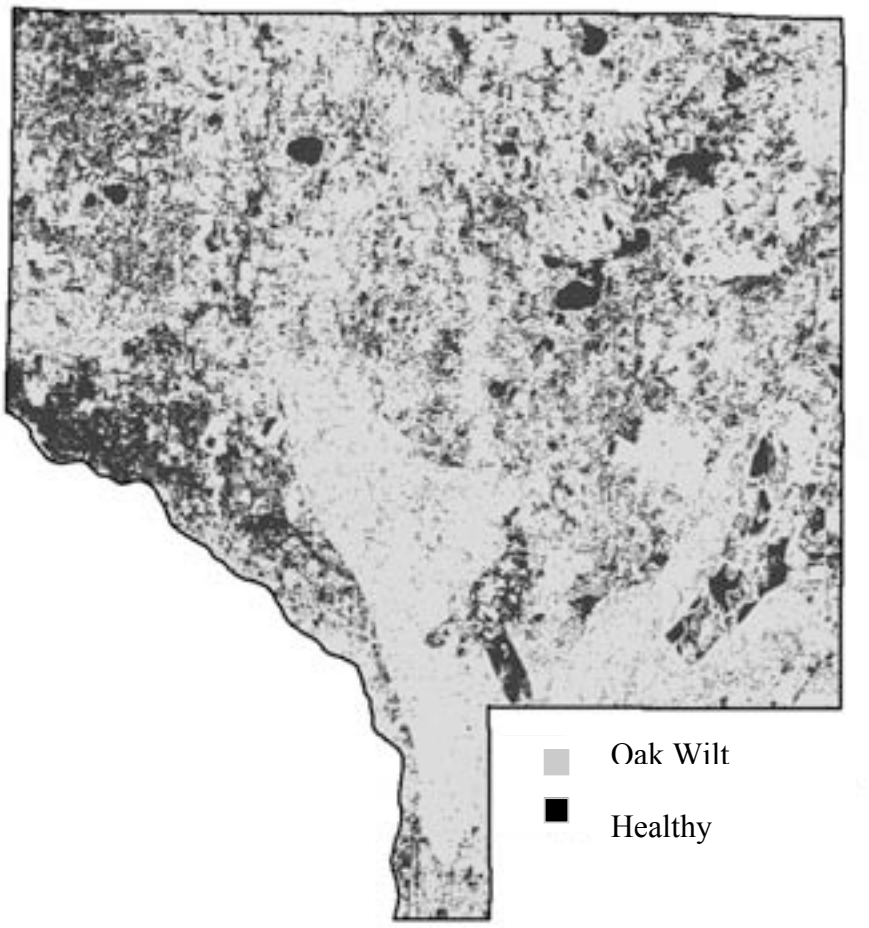


Figure 1A. Potential distribution of oak wilt with healthy oak in Anoka County, MN. The urban and non-urban models for Anoka County were merged into a single potential distribution of oak wilt grid surface according to the predicted binary output for the county.

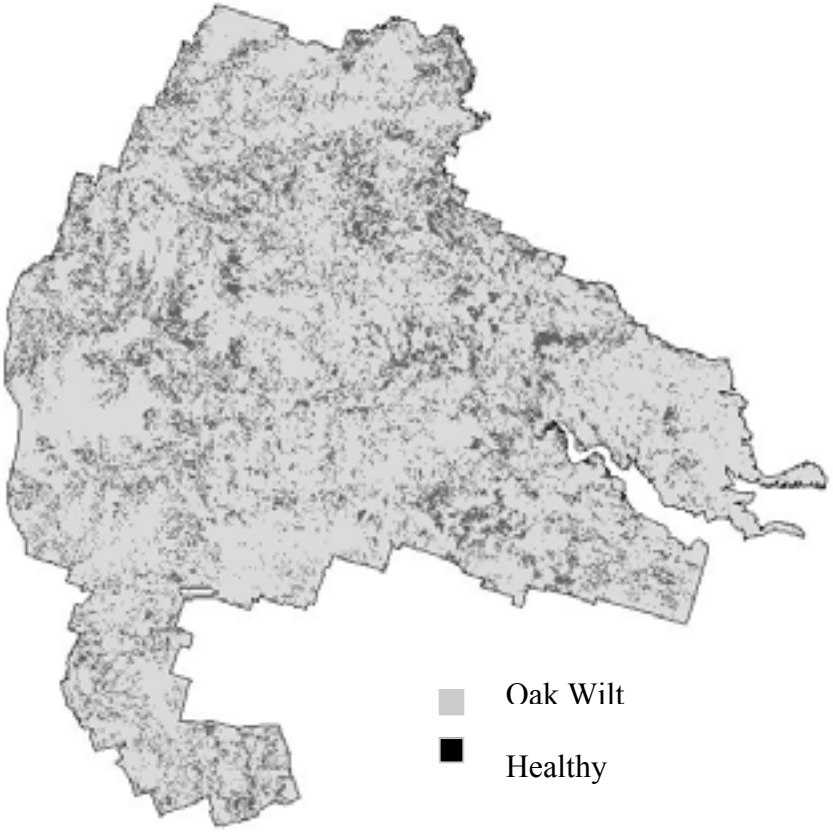
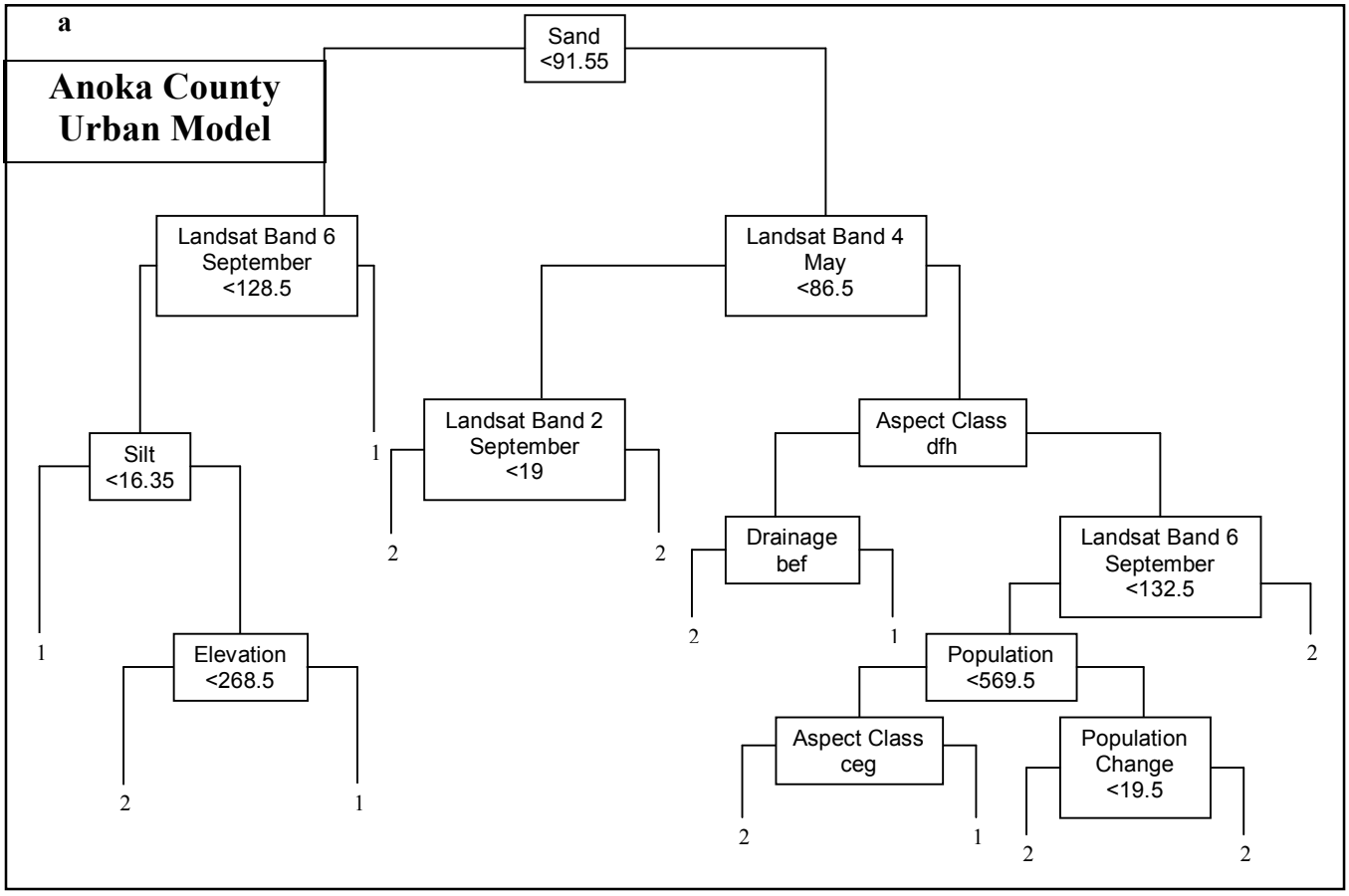
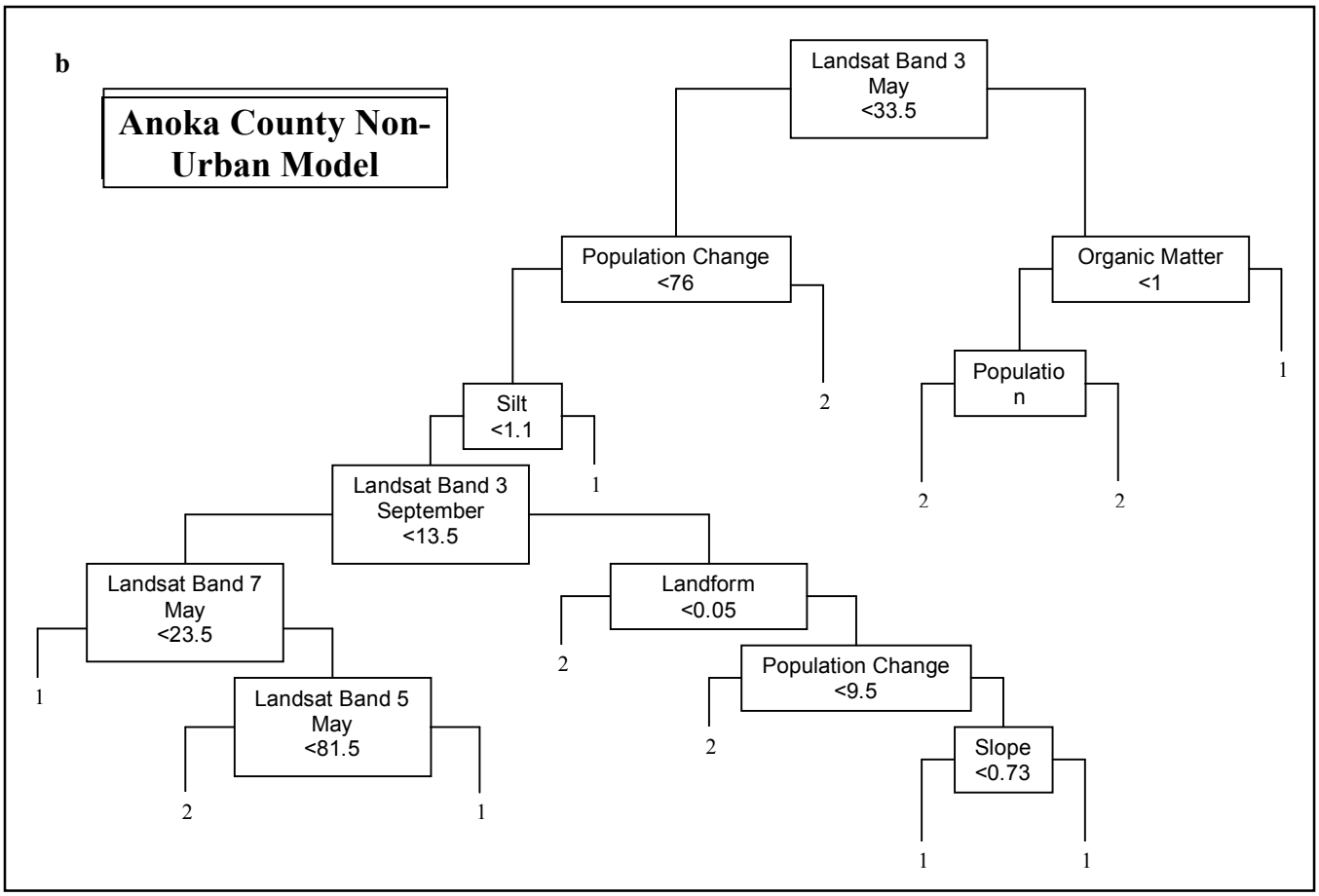


Figure 1B. Potential distribution of oak wilt and healthy oak in Fort Hood, TX.


a
Anoka County Urban Model
Sand <91.55
Landsat Band 6 September <128.5
Landsat Band 4 May <86.5
Silt <16.35
1
Landsat Band 2 September <19
Aspect Class dfh
1
Elevation <268.5
2
2
Drainage bef
Landsat Band 6 September <132.5
2
1
2
Population <569.5
Aspect Class ceg
Population Change <19.5
2
1
2
2


b
Anoka County Non-Urban Model
Landsat Band 3 May <33.5
Population Change <76
Organic Matter <1
Silt <1.1
Populatio n
Landsat Band 3 September <13.5
Landsat Band 7 May <23.5
Landform <0.05
Landsat Band 5 May <81.5
Population Change <9.5
Slope <0.73
2
1
2
1
2
2
1
2
1
2
1
1

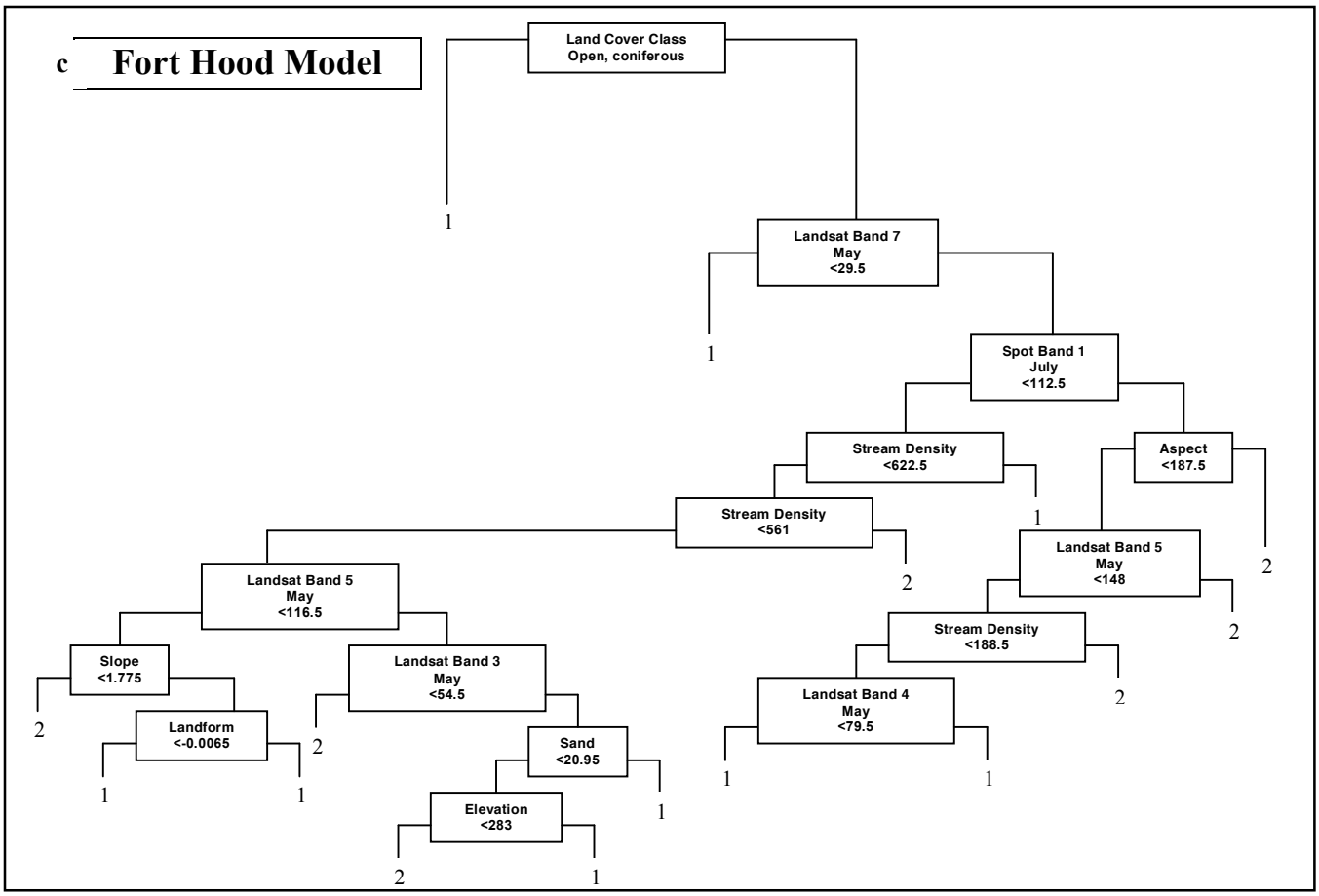


Figure 2. S-Plus classification tree output for: a) Anoka County urban model, b) Anoka County non-urban model, and c) Fort Hood model.

OAK WILT RESEARCH AT FORT HOOD: INOCULUM SOURCES AT LANDSCAPE SCALE

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ABSTRACT

Fort Hood Military Reservation supports a large population of the endangered golden-cheeked warbler (*Dendroica chrysoparia*, GCWA). Oak-juniper woodland, dominated by *Juniperus ashei* and various hardwood species, notably Texas red oak (*Quercus buckleyi*), plateau live oak (*Q. fusiformis*), and Texas ash (*Fraxinus texensis*), serves as breeding habitat for this species. Oak wilt (causal agent: *Ceratocystis fagacearum*) infects Texas red oak in central Texas and is considered a threat to the GCWA because of its potential to degrade habitat. We have used and evaluated two methods of controlling oak wilt in GCWA habitat on Fort Hood. We have tested the efficacy of basal girdling of symptomatic Texas red oak stems over a 2-year period on Fort Hood for preventing the formation of new infection centers in GCWA habitat by reducing the formation of fungal mats. Although these efforts have been successful at reducing the numbers of fungal mats in our study areas, no overall reduction in infection rates has been noted. We suspect that most new infections in our study resulted from root-to-root transmission of the pathogen, which is not controlled by basal girdling. Approximately 11.2 km of trenches have been installed over the past 4 years to control oak wilt centers in live oak in and near GCWA habitat on Fort Hood. Trenching, though limited to relatively level sites and to infection centers in live oak, has been successful at controlling the spread of oak wilt in live oak in GCWA habitat. We characterized woody species composition and structure after the passage of an oak wilt disease front and tentatively conclude that Texas red oak regeneration is adequate to replace overstory losses due to oak wilt in the absence of overbrowsing.

Key words: *Ceratocystis fagacearum,* direct control, golden cheeked warbler

Fort Hood is an 87,890-ha U.S. Army installation located in Bell and Coryell counties, Texas. Land use on the installation includes mechanized and dismounted military training as well as grazing and recreation. Fort Hood is home to two armored divisions (1st Cavalry Division and the 4th Infantry Division [Mech]), as well as associated support and aviation units. Approximately 42,000 uniformed personnel are currently assigned to Fort Hood (GlobalSecurity.org 2006). Training activities associated with these units have multiple ecological effects on the installation. Mechanized training activities involving the 2,619 tracked vehicles and 11,932 wheeled vehicles on post, as well as those of visiting units, take place throughout the installation's training areas; however, large scale off-road maneuvering is largely restricted to the West Range training areas because of terrain limitations.

Soil disturbance from vehicle traffic maintains much of the vegetation on West Range and on accessible areas of East Range in early successional stages, and accelerates sediment transport and erosion on slopes. Recovery of vegetation between traffic events is slowed by historical soil loss, compaction, periodic drought, and current grazing practices. Areas which are inaccessible to vehicular traffic and/or are not used for other reasons tend to support later successional

vegetation. These areas include slopes, hilltops, riparian areas, and smaller, more isolated training areas. Ecological effectsof training in these areas are usually caused by dismounted activities including cutting of vegetation, construction of individual fighting positions ("foxholes"), and the like.

Training also greatly influences fire frequency and timing on Fort Hood. Incendiary devices, tracers, smoke generators, and other pyrotechnic training devices provide a near-year-round source of ignition. As a result, fire frequency in this area is almost certainly higher than historical levels. Training-related wildfires are a near-daily occurrence when conditions permit, requiring the allocation of significant resources for fire suppression to protect fire-sensitive endangered species habitat as well as structures and other high-value areas. Large expanses of grassland vegetation predominate in the Live Fire Area and especially in the permanently dudded area (PD94) at its center. Woody vegetation occurs only on relatively fire-sheltered portions of the terrain in this part of Fort Hood. Conversely, areas historically dominated by grassland in the training areas of East and West Fort Hood have fewer, less intense fires because of the effects of vehicle traffic and grazing on fuels. These areas either remain in early successional vegetation (annual weeds) due to frequent disturbance or are invaded by Ashe juniper (*Juniperus ashei*) in areas where disturbance is less frequent or intense.

Fort Hood lies within the Lampasas Cut Plains, a geological region characterized by mesa topography with wide valleys separating uplands capped by limestone (Johnson 2004). Narrow canyons occur on the margins of uplands throughout this region. Vegetation in the region has changed over the past two centuries as a result of land use changes. Presettlement vegetation on Fort Hood was probably characterized by Texas red oak-shin oak-Ashe juniper (*Quercus buckleyi-Q. sinuata* var. *breviloba-Juniperus ashei*) woodlands ("oak-juniper woodland"), oak savannas, and tallgrass prairies. Because of frequent fires, woodlands were restricted to rocky slopes and mesa tops where fine fuels were less abundant (Smeins 1980).

Small inclusions of post oak-blackjack oak (*Q. stellata-Q. marilandica*) woodlands occurred on lighter textured soils atop mesas (Diamond 1997). Valleys were historically dominated by grasslands with narrow forested riparian corridors. Land use changes after settlement by Europeans, notably the expansion of row-crop agriculture, the introduction of domestic animal grazing, systematic fire exclusion, and, most recently, disturbance associated with military training, have increased the cover of woody plants, especially *J. ashei*, at the expense of herbaceous communities (Smeins 1980, Van Auken 1993).

Mature oak-juniper woodlands, dominated by Ashe juniper and a variety of oak species, now cover approximately 24,000 ha on Fort Hood. These woodlands serve as breeding habitat for the federally-listed golden-cheeked warbler (*Dendroica chrysoparia*, GCWA). Fort Hood supports the largest known population of GCWA under a single management regime; current estimates of the population are above 5,000 singing males. GCWA depend on Ashe juniper bark for nest construction material and on hardwoods for foraging substrate (Ladd and Gass 1999). Loss of either of these components makes the habitat unsuitable.

Threats to the Fort Hood GCWA population include habitat loss through land use conversion and through wildfire. Ashe juniper lacks the ability to resprout after a fire, so intense fires have the effect of greatly reducing Ashe juniper cover in these communities. Therefore, although oaks and other hardwoods resprout fairly quickly, recovery of oak-juniper woodland from wildfire to the point where it is usable for golden-cheeked warbler breeding habitat may take several decades (Reemts and Hansen 2008). Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has also been identified as a threat to GCWA because of its potential to degrade breeding habitat

by reducing the density of Texas red oaks. The US Fish and Wildlife Service has stated that oak wilt monitoring, research, and control should be a part of management activities for GCWA on Fort Hood (USFWS 2005). However, the extent and seriousness of this threat is unknown. In areas where excessive herbivory or other factors prevent regeneration of oaks (Van Auken 1993, Russell and Fowler 2002), the loss of mature Texas oaks to oak wilt would appear to present a real threat to habitat quality for GCWA. It is unclear whether oak wilt would similarly threaten GCWA habitat in areas where sufficient oak regeneration is occurring.

Oak wilt infects a broad range of oaks, but red oaks (subgenus *Quercus*, section *Lobatae*), including Texas red oak, are highly susceptible, while white oaks (subgenus *Quercus*, section *Quercus*) are generally more resistant and often recover from infection (Appel 2001). Oak wilt spreads over short distances through xylem connections between trees, either via common root systems or through natural root grafts. From a single infected tree the disease typically spreads through root systems to neighboring trees to form a disease center (Appel 2001). Long-distance transmission of oak wilt occurs primarily by insect-vectored transmission of spores (either conidia or ascospores) from fungal mats which form under the bark of infected, dying red oaks. Fungal mats typically form on a small proportion of red oaks during late winter and early spring which were symptomatic the previous summer. Beetles of the family Nitidulidae have been most commonly implicated as vectors (Appel, Anderson and Lewis 1986, Appel, Kurdyla and Lewis 1990, Juzwick 2001). These beetles are attracted to the fungal mats from which they transport spores to uninfected trees.

Oak wilt is common on Fort Hood, both in Texas red oak and in plateau live oak (*Quercus fusiformis)*. Infection centers in both species threaten GCWA habitat, both because plateau live oak is an important component of the vegetation in many areas of GCWA habitat on the installation and because of the possibility that the disease will spread from plateau live oak to Texas red oak through interspecific root grafts. The oak wilt pathogen moves in both species via root connections, producing spreading disease centers which remain active for several years. The conditions on Fort Hood, including the presence of an endangered bird species which depends on susceptible oaks for its survival, as well as the unique mix of land uses that Fort Hood hosts, pose a series of questions for managers of GCWA habitat, and in particular, oak wilt in that habitat.

First, we wanted to know whether recommended methods for controlling and reducing oak wilt in endangered species habitat were effective. Basal girdling has been recommended for control of oak wilt fungal mat formation for many years (Morris 1955, Gillespie, Shigo and True 1957, Texas Oak Wilt Information Partnership 2007). The Nature Conservancy has been applying basal girdling to Texas red oak in GCWA habitat for the past 4 years in an attempt to limit the production of fungal mats in these areas. We tested the efficacy of this practice in Texas red oak populations on Fort Hood (Greene, Reemts and Appel 2009).

Second, at a larger scale, we have attempted to answer the question whether reducing fungal mat numbers actually decreases the amount of oak wilt in a stand in subsequent years. This effort will also be reported in Greene, Reemts and Appel (2009). Together, the answers to these questions will indicate whether oak wilt management on Fort Hood can be effective at preventing GCWA habitat degradation.

Finally, given that oak wilt is common in GCWA habitat on Fort Hood, it is important to characterize the vegetation regeneration after the Texas red oak overstory is removed by oak wilt. Understanding how oak wilt modifies the vegetation will provide insight into whether the

modified vegetation will be suitable habitat for GCWAs. We have made preliminary measurements in an attempt to answer this question.

EFFICACY OF BASAL GIRDLING

To test the efficacy of basal girdling to prevent fungal mat formation in oak wilt-infected, symptomatic Texas red oak stems, we conducted a study over three years in which randomly selected experimental units in three blocks were treated by either tagging and girdling all symptomatic Texas red oaks in two successive late-summer periods (2004 and 2005) or by tagging alone, without girdling. Experimental units ranged in size from 62 to 85 ha in size. We followed treatments with assessments in the early spring of the next growing season to determine presence and number of fungal mats. We tested two hypotheses: 1) that late summer basal girdling of symptomatic Texas oak reduces the formation of fungal mats in the following spring, and 2) that late summer basal girdling reduces the incidence of oak wilt symptom development during late summer of the year following the treatment. Procedures for this study are described in Greene, Reemts and Appel (2009).

Basal girdling significantly reduced the probability that a stem would produce fungal mats. The mechanism by which this treatment works is uncertain; evidence in the literature indicates that removing the bark may cause the dying tree to dry out before mats can form (Wilson 2005), or that mechanical damage to the bark introduces competing fungi (in particular, *Hypoxylon* spp.) which colonize the tree and competitively exclude *C. fagacearum* (Tainter and Gubler 1973, 1974). We also noted that stem diameter had a significant effect on the likelihood that a stem would form a fungal mat. Since smaller stems dry out more quickly, this piece of evidence seems to support Wilson's (2005) theory. However, we also observed abundant *Hypoxylon* fruiting structures on many treated stems and noted that these stems rarely formed *Ceratocystis* fungal mats.

When we surveyed our study areas after 1 and 2 years of treatment, we could not detect any treatment effect on overall incidence of oak wilt at the stand level. We suspect that basal girdling may not appreciably affect subsequent infection rates because of the comparative rarity of insect-mediated, spore transmission (which basal girdling could be expected to control) compared with transmission through root connections (which is not known to be affected by girdling). This finding led us to recommend that basal girdling be used sparingly in Texas red oak and only in conjunction with measures to control root-to-root transmission.

TRENCHING

Trenching with a rock saw to a depth of 1.5 m is widely practiced in the Edwards Plateau and has been demonstrated to be effective at controlling oak wilt infection centers in plateau live oak. However, this method of oak wilt control has some important limitations that restrict its use on Fort Hood. First, because of the machinery required to dig the trench, trenching is only feasible on level and gently sloping areas. Since many of the slopes on which GCWA habitat occurs on Fort Hood are steep and rugged, trenching is not an option there, both because of the machinery's limitations and because soil disturbance on steep slopes has the potential to cause erosion. In addition, trenching is quite expensive, and creates habitat breaks which may degrade habitat quality.

Despite these limitations, we have installed 11.2 km of trenches over the past 4 years at Fort Hood. All of the trenches have been deployed either in or near GCWA habitat around disease centers in plateau live oak. We have tried to keep the trenching machine on existing trails, even

if that meant sacrificing a few more live oaks to the disease center in some cases. Trenches have been largely effective at stopping the spread of *Ceratocystis* in plateau live oak stands; there has been only one breakover during the 4-year history of the program. We were able to enclose the resulting outbreak with a second trench the following year.

REGENERATION IN OAK WILT CENTERS

After passage of an oak wilt disease front in oak-juniper woodland, overstory cover is reduced and woody regeneration is released. This is especially true where fire or human disturbance has previously removed the juniper component, leaving coppice-regenerated hardwoods. Regeneration inside oak wilt centers in these areas is of interest for two reasons. First, successful Texas red oak seedling regeneration would perpetuate this component of the woodland into the next stand. Second, although work is underway to study the effect of fire on oak-juniper woodlands (Reemts and Hansen 2008), it is unknown how fire and oak wilt interact with this vegetation type.

In 2006, we examined vegetation on either side of a moving disease front in stands which consisted largely of Texas red oak sprouts of fire origin. Fire scar and tree ring analysis indicated that the stands originated after a 1988 fire and were thus 18 years old at the time we examined them. We measured overstory and regeneration in 3 pairs of nested plots systematically located behind and just ahead of active oak wilt disease fronts on Fort Hood. We estimated that Texas red oaks in the plots inside the oak wilt centers had died 2 to 4 years before the data were collected, based on the state of decomposition of the snags.

Before disease front passage, the basal area in these stands was 75% Texas red oak; the balance was composed primarily of relatively shade-tolerant understory species. Most common were redbud (*Cercis canadensis* var. *texensis*), Carolina buckthorn (*Frangula caroliniana*), and dogwood (*Cornus drummondii*) (Fig. 1). Overstory canopy cover was nearly 100% Texas red oak. Total basal area was 18.6 m^2/ha. Two to 4 years after passage of the disease front, total basal area had been reduced to 4 m^2/ha, basal area of Texas red oak was reduced to 38% of the total, and small components of Ashe juniper, shin oak, and flameleaf sumac (*Rhus lanceolata*) had reached breast height and thus were contributing to basal area (Fig. 2).

Regeneration of all species was much more abundant 2 to 4 years after disease front passage. In particular, Texas red oak regeneration increased nine-fold from 1,326 stems/ha, all shorter than 30 cm, before disease front passage to 12,335 stems/ha after front passage; 45% of these stems were taller than 30 cm (Fig. 3). This finding is in contrast to that of Russell and Fowler (2002), who reported a dearth of Texas red oak regeneration due to overbrowsing by white-tail deer (*Odocoileus virginianus*). Overbrowsing by deer is not generally observed on Fort Hood because deer populations are maintained at relatively low levels.

Although these data are very preliminary, two observations seem warranted at this time. First, it is apparent that Texas red oak will make up a significant fraction, but probably much less, of the next stand. Second, although the regenerated stand is very young, it appears to be more diverse than the fire-origin coppice-regenerated Texas red oak stand. It remains to be seen whether this increased diversity will be maintained as the stand matures.

MANAGEMENT IMPLICATIONS

Based on the results of our research and our experience with operational oak wilt control measures on Fort Hood, we note the following:

- We have discontinued the use of basal girdling, by itself, as a control measure for oak wilt in Texas red oak in GCWA habitat because it appears not to be effective at reducing infection rates in subsequent years. In cases where root transmission of the oak wilt pathogen is controlled by mechanical or other means, basal girdling may be an effective method of reducing the risk of insect-mediated spread.
- Trenching is useful for containing oak wilt centers on level to gently sloping terrain. Operational concerns limit the use of this method on mesa slopes, where the best GCWA habitat occurs.
- There does not appear to be any shortage of Texas red oak regeneration in oak wilt centers in stands composed mostly of Texas red oak (i.e., where the disease results in the death of most of the overstory). It is likely therefore that Texas red oak will comprise a significant fraction of the resulting stand. We have not studied regeneration in centers where Texas red oak is a small fraction of the overstory, and therefore much of the canopy remains intact after passage of the disease front.

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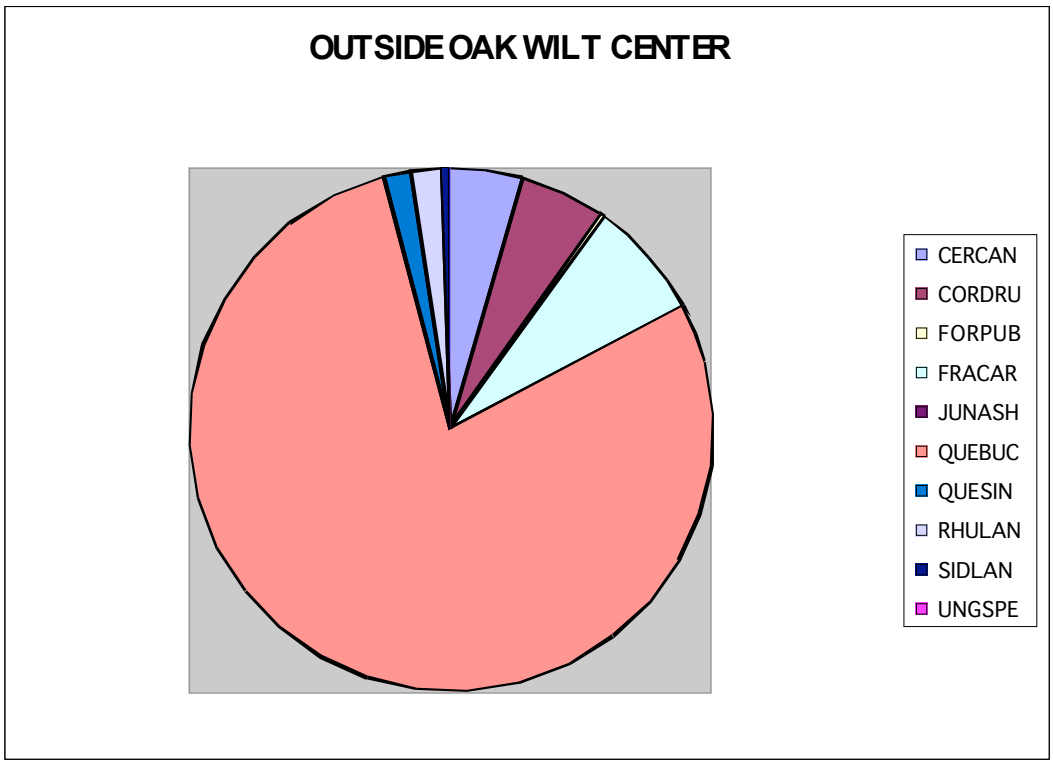

Figure 1. Relative basal area of woody stems, by species, on three, 0.01-ha plots outside an oak wilt disease center on Fort Hood, Texas. Species codes are: CERCAN=*Cercis canadensis*; CORDRU=*Cornus drummondii*; FORPUB=*Forestiera pubescens*; FRACAR=*Frangula caroliniana*; JUNASH=*Juniperus ashei*; QUEBUC=*Quercus buckleyi*; QUESIN=*Quercus sinuata*; RHULAN=*Rhus lanceolata*; SIDLAN=*Sideroxylon lanuginosum*; UNGSPE=*Ungnadia speciosa.*

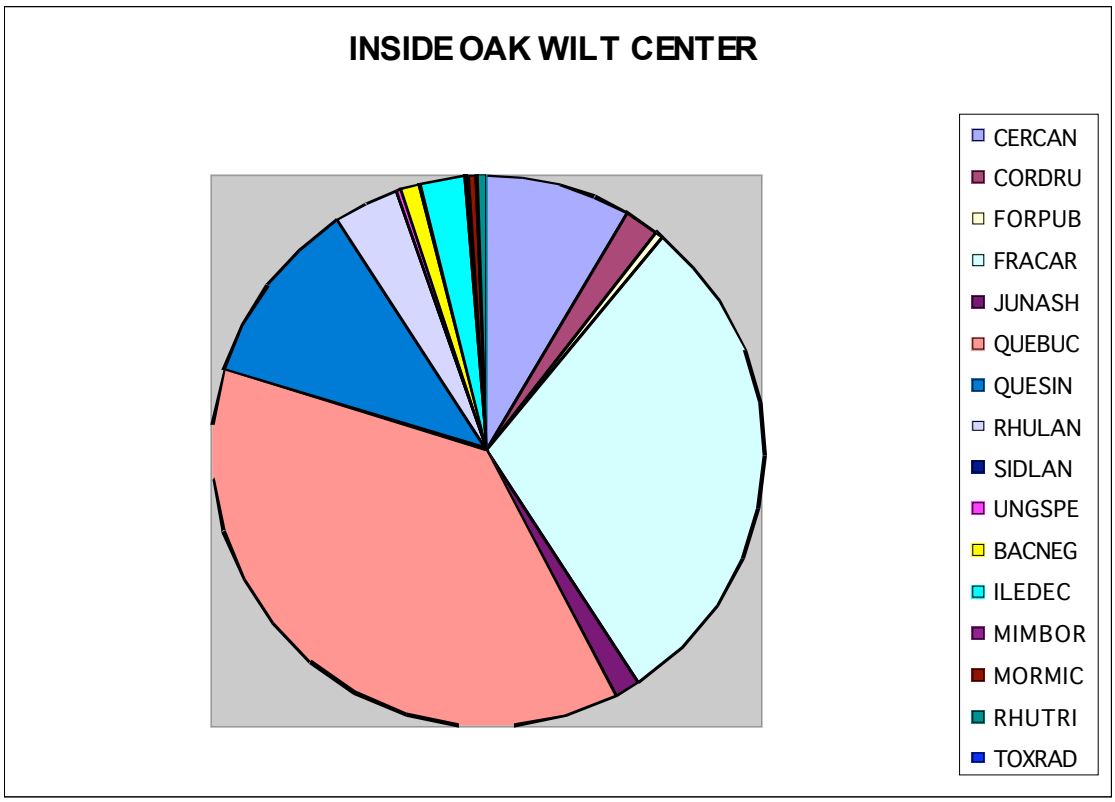

Figure 2. Relative basal area of woody stems, by species, on three, 0.01-ha plots inside an oak wilt disease center on Fort Hood, Texas. Species codes are: CERCAN=*Cercis canadensis*; CORDRU=*Cornus drummondii*; FORPUB=*Forestiera pubescens*; FRACAR=*Frangula caroliniana*; JUNASH=*Juniperus ashei*; QUEBUC=*Quercus buckleyi*; QUESIN=*Quercus sinuata*; RHULAN=*Rhus lanceolata*; SIDLAN=*Sideroxylon lanuginosum*; UNGSPE=*Ungnadia speciosa*; BACNEG=*Baccharis neglecta*; ILEDEC=*Ilex decidua*; MIMBOR=*Mimosa borealis*; MORMIC=*Morus microphylla*; RHUTRI=*Rhus trilobata*; TOXRAD=*Toxicodendron radicans*.

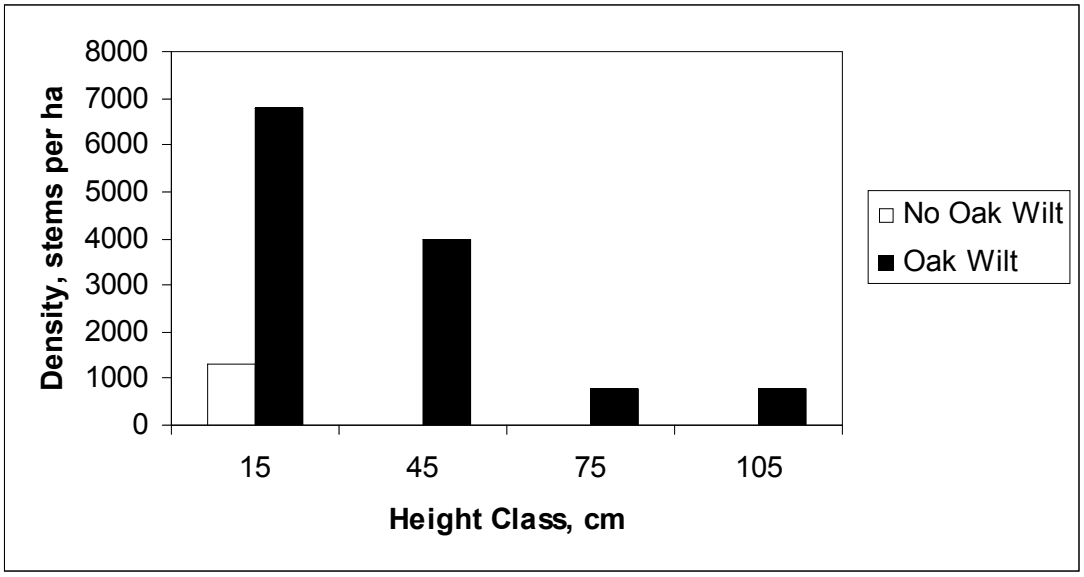

Figure 3. Texas red oak regeneration by 30-cm height class before passage of an oak wilt disease front, and 2-4 years after passage, in oak-juniper woodland on Fort Hood, Texas.

OAK WILT RESEARCH AT FORT HOOD AND IMPACT ON GOLDEN CHEEKED WARBLER NESTING SITES

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ABSTRACT

Understanding how oak wilt can impact management activities has recently become an issue at Fort Hood, TX. Fort Hood is home to an endangered bird species, the golden cheeked warbler (GCW), *Dendroica chrysoparia*. The GCW uses juniper trees (*Juniperus ashei*) for building nests and feeds on Lepidoptera that exist in oak species. It's become a concern whether the oak wilt pathogen is affecting the GCW habitat, nesting and feeding activities. Two surveys were conducted in 2001 and 2002-2003. The 2001 survey used IKONOS 1-meter pan-sharpened satellite imagery for photo interpretation of mortality centers within the post perimeter. In 2002-2003, field surveys were conducted in five distinct categories (GCW/OW, non-GCW/OW,

GCW/non-OW, and non-GCW/non-OW, GCW/NS). Decision tree analysis was used to determine important characteristics of GCW nesting habitat using collected field and independent data. Results of this study will help with management conflicts that occur between oak wilt control and conservation of endangered species habitat.

Key words: *Ceratocystis fagacearum, Dendroica chrysoparia*

The destructive tree fungus *Ceratocystis fagacearum* (Bretz) Hunt has been well documented throughout the U. S. In Texas, hundreds of acres and thousands of trees have been destroyed by this pathogen (see website at http://www.texasoakwilt.org). Not until recently has the impact of oak wilt on an endangered species and its habitat been evaluated. The endangered species of concern is the golden cheeked warbler (GCW, *Dendroica chrysoparia*). In 1990, at the time the GCW was designated an endangered species, a formal management plan for restoration of the GCW was proposed. This included research on the impact of oak wilt on the GCW habitat (Keddy-Hector 1992). Not until this study has this research initiative been addressed. The present study was conducted on the Fort Hood military base to assess the influence of oak wilt on this endangered species habitat and nesting site locations and to see if costly control measures for oak wilt are advised. This will be useful for managers responsible in making oak wilt management decisions.

DESCRIPTION OF FORT HOOD, TEXAS

Fort Hood is located in the hill country of central Texas covering portions of Bell and Coryell counties and is one of the largest army installations in the United States. Fort Hood covers 88,500 hectares (217,000 acres) and consists of a mix of grassland, open savannas, hardwood thickets, and dense oak-juniper stands (Dearborn et al. 2001). It also has constant ongoing, destructive, large-scale landscape activities. Fort Hood houses two full armored divisions (1st Calvary Division and the 4th Infantry Division), conducts full military training operations including large-scale troop and vehicle movements, allows cattle grazing under lease through cattlemen's associations, houses areas for public recreation and operates under the auspices of the endangered species act.

Fort Hood supports significant breeding populations of two endangered species; blacked-capped vireo (*Vireo atricapilla*) and the golden cheeked warbler and lies at the intersection of two Nature Conservancy ecoregions. The Nature Conservancy has recognized Fort Hood as a priority site and has been working with Fort Hood's endangered species management program since 1993 to lessen the impact on the fragile forest ecosystem that the various activities at Fort Hood have (see Nature Conservancy's website http://www.nature.org, Greene and Reempts, this proceedings).

INTERACTION OF GOLDEN CHEEKED WARBLER AND OAK WILT

The golden cheeked warbler is a migratory songbird that arrives in central Texas in early spring for breeding and leaves to its post-breeding grounds in Central America and southern Mexico in mid- to late June (Ladd and Gass 1999). The breeding and nesting requirements of the GCW are particularly dependent upon certain characteristics of the oak/juniper savannas of central Texas (Kroll 1980). GCW habitat is dependent on Ashe juniper (*Juniperus ashei*) and a variety of oak species that are dominated by Texas red oak (*Q. buckleyi*) and shin oak (*Quercus sinuata*) (Kroll 1980, Wahl et al. 1990). The GCW uses the shedding bark from mature Ashe junipers for

nesting material and forages on lepidopteron insects that exist in high populations in oak canopies (Smith 1916, Simmons 1924, Pulich 1976, Kroll 1980, Ladd 1985 and Wahl et al. 1990). The GCW primarily nests in Ashe junipers but nests have also been found in Texas red oak, post oak (*Quercus stellata*), Texas ash (*Fraxinus texensis*) and live oak (*Quercus fusiformis*) trees in Fort Hood (Hayden et al. 2001).

The GCW is attracted to more mesic areas within the juniper-oak complex, such as canyons and seepy hill sides where deciduous hardwood vegetation is more abundant (Hayden et al. 2001). Fort Hood has designated 21,850 ha (53,991 acres) or 24.7% total post as GCW habitat (Dearborn and Sanchez 2001). Urbanization, fragmentation of breeding habitats for agricultural purposes, and parasitism are the primary reasons given for the decline in GCW numbers throughout its northern range (USFWS 1990, Moses 1996). Other disturbances on the GCW habitat such as oak wilt need to be considered and require further study to determine if this disease of native oaks is partly responsible for the decline in the numbers of GCW.

Ceratocystis fagacearum, the fungus that causes oak wilt, is a destructive pathogen that kills hundreds of red and live oaks every year in Texas. Oak wilt is caused by a vascular pathogen that spreads through interconnected root systems in live oaks. In red oaks, a brief saprophytic phase is supported where means of overland spread occurs. These two means of spread, above and below ground, greatly influence the spatial pattern and rate of spread of this pathogen and can have a strong effect on the forest ecosystem. Oak wilt management also needs to be considered when determining how to control the pathogen on a large landscape scale. Large landscape control techniques would consist of destroying diseased red oaks and trenching to break up the root systems of live oaks infected with oak wilt (Appel 1995). These techniques are extremely costly and cause great disturbances to the area when applied. Natural resource managers must have a thorough understanding of the epidemiology, impact on the GCW habitat and biology, and predict long-term consequences of their actions. This is becoming increasingly difficult especially when managers must contend with complex multiple land-use objectives such as those that exist on Fort Hood. This research project provides the ability to predict the incidence and intensity of oak wilt and how it impacts the GCW habitat, which provides a valuable tool in the decision-making process.

METHODS AND MATERIALS

2001 Field Survey

To determine how oak wilt affects GCW habitat, the number of oak wilt centers on Fort Hood needed to be determined. In 2001, a photogrammetric survey followed by a ground survey of the delineated sites was conducted. IKONOS 1-meter pan-sharpened satellite imagery was obtained for Fort Hood, including a 1-mile (1.6 km) buffer around the post boundary (Pacific Meridian Resources, Emeryville, CA 94608). The imagery was co-registered to Orthophoto Quarter Quadrangles (DOQQ's) using the geographic information system ArcView (ESRI, 380 New York St., Redlands, CA 92373). Survey lines at 330 m spacing were transposed onto the satellite imagery. A trained technician selected the mortality areas on the map that were representative of tree mortality, with attempts to exclude as best as possible mortality areas caused by fire, brush-clearing, and unknown sources. Oak wilt mortality occurs in expanding centers so differentiating between oak wilt and other causes of mortality is relatively simple. Once the mortality polygons were completed, they were overlaid onto the imagery to be used for ground truthing. A random sample of 10% of the photointerpreted polygons was selected for diagnosis.

Oak wilt was diagnosed according to recognized symptoms of the disease in the field and by laboratory isolation of the pathogen when necessary (Appel 2001).

2003-2004 Survey and Classification Tree Analysis

One of the next goals was to characterize typical GCW nesting- and habitat sites and the impact of oak wilt. This goal was part of a larger project conducted in cooperation with the USDA Forest Service Forest Health Technology Enterprise Team, (FHTET, Ft. Collins, CO), which used the methodology of binary classification and regression tree analysis (CART) to model and predict oak wilt incidence and severity (see Downing et al., this proceedings). Tree-based modeling is an exploratory technique for uncovering structure in data (Clark and Pregibon 1992). Classification trees can explain the variation of a single response variable by one or more exploratory variables which are useful for ecological data that is often multifaceted, unbalanced and contains missing values. The result of this non-parametric technique is a classification tree used to explain the variation of a dependent or response variable by a collection of independent or explanatory variables (Baker et al. 1993, De'ath and Fabricius 2000).

The tree is constructed by repeatedly splitting the data into two mutually-exclusive groups that are each as homogeneous as possible but while also keeping the tree reasonably small (De'Ath 2000). To keep the trees as accurate as possible, a cross validation procedure is performed. This looks at the independent variables from the tree and calculates the amount of error produced by iteratively combining the independent variables. The result is a plot of the number of terminal nodes and misclassification error. The original tree is then pruned to the best model with the greatest number of terminal nodes and the least amount of classification error. FHTET used the dependent variable of presence or absence of oak wilt, whereas for this paper the presence or absence of nesting sites was used, but the field data gathered could be used for both analyses. Ancillary data used in the analysis was obtained from the Natural Resources Management Branch (ARMY) office at Fort Hood.

Sample plots (n=137) were randomly selected using a Sample Points Generator (SPGen), an ArcView application, from four land categories: 1) GCW habitat, non-oak wilt, 2) GCW habitat, oak wilt, 3) non-GCW habitat, oak wilt, and 4) non-GCW habitat, non-oak wilt. The fifth category of nesting sites (GCW/NS) that were known to be occupied by nesting pairs in 2002-2003 was subsequently added as an additional dependent variable for this paper's CART analysis. Independent variables for the model were derived from each of the four bands of 2003 SPOT 10 and each of the seven bands of Landsat TM satellite imagery and the eleven 30m grid themes (slope, elevation, aspect, soils, distance to roads, road density, distance to streams, stream density, distance to lake, forest savanna, and landform)

The grid themes were created by using the imagery bands and the ERDAS Imagine Software Grid Export function (ERDAS, Inc., ERDAS Imagine V8.5.1002. Atlanta, GA). Surveys were conducted by the USDA Forest Service, the Nature Conservancy, the Texas Forest Service, and Texas A&M University in 2003 - 2004. The cluster plots were distributed throughout the five sampling categories and each plot consisted of a 20m x 20m fixed square plot subdivided into 4 10m x 10m sub-plots. Data collected for each plot and subplot consisted of tree diameters, tree species identification, symptom development of infected trees, dominant overstory and understory species, and average tree height.

The classification tree was fitted to the spatial information database using S-PLUS © statistical software package (Insightful Corp, Seattle, WA 98109). Twenty-two independent variable grid themes and twenty-five data categories were used to construct the classification tree

for comparing the nesting site data with the data from the GCW habitat with no oak wilt present (GCW/non-OW). This comparison was run with three sets of data: grid theme and field data (total data), grid theme data only (independent data), and field data only (field data).

An analysis of the total survey data was conducted as well to determine significant differences among the category types. These consisted of analysis of stand characteristics such as species frequency, size class differences, tree density, juniper to oak ratio, and age of junipers. Age of junipers was determined based on the regression formula presented by Kroll (1980) which used diameter measurements to determine the age of Ashe junipers. Multi-response Permutation Procedures (MRPP) and non-metric multidimensional scaling (NMS) were used by PC-ORD © for Windows version 4.01 (McCune and Mefford 1999) to determined differences of species composition and tree diameters among the 5 categories. Logarithmic transformation of the data was applied to perform linear regressions using proc glimmix in SAS © version 9.1 (SAS Institute Inc., Cary NC 27513) to determine differences in stand structure. Linear regressions tested yes, no relationships on the data (e.g., whether sites were oak wilt or GCW, and if sites were GCW or nesting sites)

RESULTS

2001 Field Survey

Photo interpretation of the IKONOS satellite imagery for tree mortality for the post plus the one-mile (1.6-km) buffer and the post without the buffer revealed 1,164 and 638 mortality polygons respectively (Fig. 1). The 10% sample, 119 plots, revealed that 60 (82%) mortality polygons within the post only were caused by oak wilt. Other minor factors that were attributed to tree death consisted of military ops, brush piles, blow downs, and fire. Of the 73 photo-interpreted sites that fell within the post perimeter, 12 (16%) were within GCW habitat and 7 (12%) were oak wilt. Six additional oak wilt centers were within 100 feet of golden cheeked warbler habitat. Extrapolating for the entire post, 9% of the mortality centers were estimated to be oak wilt within GCW habitat.

2003-2004 Field Survey

Stand characteristics. Nesting sites (GCW/NS) had the highest stand density (n=1296) (Table 1). Nesting sites (65%) and GCW habitat only (GCW/non-OW) (77%) had the highest juniper composition, compared to non-GCW/OW (14%) (Table 1). The number of live oak stems was considerably lower in GCW/NS (n=34) and GCW/non-OW (n=38) compared to non-GCW/OW (n=260) (Table 2). The juniper to oak (J:O) ratio varied among the plot types, but GCW nesting sites and GCW/non-OW had the highest J:O ratio. Sites that contained only oak wilt outside of the GCW habitat had the lowest juniper to oak ratio (Table 1).

The numbers of mature Ashe juniper were higher in GCW habitat and nesting sites when compared to non-GCW habitat (Table 3). Nesting sites and GCW/non-OW sites tended to have more trees in the mature age classes (>50 years) then did the GCW/OW sites.

Linear regression analysis was performed on the five categories (GCW/NS, GCW/non-OW, GCW-OW, non-GCW/non-OW, non-GCW/OW). Significant differences ($p<0.0450$) in diameters in nesting sites when compared with GCW habitats were found. MRPP analysis on the interaction of species between the five categories revealed that there were distinct differences ($p=0.0000$) among categories with regard to species composition. Species composition was further tested by NMS analysis which revealed the species of most importance for nesting sites were juniper and shin oak (Fig. 2).

Classification tree model. This model was used for the survey data on nesting sites (GCW/NS) in 2004 and from the data collected in 2003 and 2004 from one of the sample plot categories (GCW/non-OW) (Figure 3). The dependent variable was the nesting sites which were assigned the value of 1 while the GCW/non-OW sites were assigned the value of 0. This analysis included both the field and the independent data (total data). The resulting classification tree had an accuracy of 98.2% with 8 terminal nodes (Fig. 4). Discriminating variables included road density, Landsat band 6, elevation, distance to roads, and Spot band 3. Low road density accounted for most of the variance (62%) in the nesting site locations, followed by Landsat band 6 (43%), elevation greater than 247 m (30%), distance to roads (6%), and spot band 3 (3%). Based on the model, the best and most favorable nesting sites were at locations with a low road density (<586.5), an elevation greater then 247.5 m, and distance to roads of less then 91.5 m. When road density was high (>969.5 m) and distance to roads greater than 91.5 m, the probability of GCW nesting site habitat was low.

DISCUSSION

Numerous oak wilt sites exist on the Fort Hood post and some fall within and near golden cheeked warbler habitat. Photointerpretation was a reasonably accurate technique for identifying oak wilt mortality centers on satellite imagery. Though some error existed, this process could be more refined and accurate with time and experience in photo interpretation. Oak wilt proved to be the dominant cause of tree mortality on the post. Fire could be considered a greater mortality feature, even more so then oak wilt, in the specific locations where it occurred. The comparison of fire and oak wilt on stand type should be considered for future study.

Characterizations of golden cheeked warbler habitat and nesting sites have been previously described in the literature (Pulich 1976, Kroll 1980, Dearborn et al. 2001). Preferred GCW habitat consists of a climax forest type comprising mature dominant Ashe juniper and a variety of oak species. Ashe junipers are considered mature when they are greater then 50 years old (Kroll 1980). In our survey plots, both the nesting sites and GCW habitat fulfilled these requirements for mature Ashe juniper. Similar numbers of Ashe junipers in all age classes were also found in GCW/non-OW and GCW/NS. When oak wilt was present, there were fewer junipers in general and fewer junipers in the mature category, suggesting that stands with GCW have a different stand structure than those with oak wilt.

Dearborn and Sanchez (2001) found that GCW nest patches in Fort Hood were found in high densities of small, young junipers. Kroll (1980) found that GCW habitat in the Meridian state recreation area had high numbers of small Bigelow oak (*Quercus durandii breviloba*). A significant difference between our plots was the presence of numerous small-diameter shin oak in nesting sites when compared to GCW habitat. The presence of these small diameter shin oaks and the higher ratio of junipers to oaks would coincide with what Dearborn and Sanchez (2001) and Kroll (1980) found for preferred GCW habitat and nesting sites. Sites where oak wilt was present had much lower densities of trees, suggesting a different stand structure exists for GCW nesting site habitat and oak wilt locations.

Previous research on juniper and hardwood composition for GCW habitat has shown that good GCW habitat had a juniper composition of 14-50% and a hardwood composition of 20-70% (Hayden 2001). Good habitat at Meridian State Recreation Area was reported to have 52% Ashe juniper, 33% shin oak and 5% Texas oak (Kroll 1980). Our results showed that GCW/NS habitat in Fort Hood had 65% juniper and 35% hardwood composition. Habitat containing only

GCW habitat had the same trend of having a higher percentage of junipers and low percentage of hardwoods. Interestingly, when oak wilt is present, the composition of juniper decreases and oaks increases, which would preclude different stand structures for these two habitat types (GCW nesting site and oak wilt locations).

Live oaks dominated the oak wilt sites whether GCW habitat was present or not, whereas in GCW nesting sites the composition of live oaks was reduced. Sites where live and red oaks are both dominate were characteristic of oak wilt sites with no GCW. To support a high incidence of oak wilt requires a live oak host type to support root to root spread and red oaks to provide the inoculum source in the form of fungal mats. The proportion of red oaks is fairly consistent among the habitat types so the amount of inoculum that would be produced could be considered uniform.

From the results, it appears that live oaks have different site requirements then the habitat found in GCW sites and thus the threat of oak wilt in critical habitat is less then predicted. This is further supported by NMS analyses which determined that juniper and shin oak were the most important species in nesting sites and live and red oaks were not present in significant numbers. This again supports the conclusion that GCW nesting habitat and sites with oak wilt have different stand structures. This stand structure in which shin oak is one of the dominate stand components of GCW nesting habitat was found for Fort Hood; other areas where GCW exist in Texas may have a different major oak component. Further research needs to be completed at these other locations (i.e., Balcones Canyonland Preserve and Meridian State Park Recreation Area).

The classification tree analysis proved to be an excellent technique for determining the factors that are influential in distinguishing GCW nesting sites. Our tree revealed that preferred nesting site locations would be in areas with low road density and high elevations. These results are consistent with research that shows GCW prefers large blocks of unfragmented tracks of land (Ladd 1985, Moses 1996), though this belief is contentious. Conflicting opinions exist that GCW co-evolved as an edge species that inhabits the interfaces between grassland and juniper-oak stands (Kroll 1980). Kroll found when large homogenous blocks of land exist, GCW territories usually occur along the outer edges and habitat should consist of Ashe junipers along streams and hill crests. The expansion of oak wilt centers that cause patches and create edge effects needs to be further studied to see how the disease relates to GCW besides loss of host type.

Oak wilt causes many forms of disruption such as creating patches, edge effects, changing stand structure (tree composition, size classes, and density). More research is needed on the effects of oak wilt and GCW habitat and nesting locations so land managers can be more confident in making critical decisions regarding the need to control this disease. The amount of disruption created by implementing the control techniques also needs to be taken into consideration.

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Table 1: Stand structure of the five sample plot categories for the 2003-2004 survey on Fort Hood, TX.

Category[a]	Tree /Hectare	% Juniper	% Hardwood	J:O Ratio
GCW/non-OW	886	77	23	6.57:1
GCW/OW	639	56	44	1.66:1
Non GCW/ non-OW	90	45	54	2.48:1
Non GCW OW	570	14	86	0.24:1
GCW/NS	1298	65	35	3.16:1

[a] GCW/non-OW = Golden cheeked warbler no oak wilt,
GCW/OW = golden cheeked warbler with oak wilt,
Non GCW/non-OW = no golden cheeked warbler, no oak wilt,
Non GCW/OW = no golden cheeked warbler with oak wilt,
GCW/NS = golden cheeked warbler nesting site.

Table 2. Total number of trees and proportion of total for the five tree species sampled in the five sample plot categories for the 2003-2004 survey on Fort Hood, TX.

Species[b]	GCW/OW	GCW/ non-OW	non-GCW/ non-OW	non-GCW/ OW	GCW/NS
DH	55 / 0.11	115 / 0.12	42 / 0.37	190 / 0.26	185 / 0.15
J	301 / 0.62	762 / 0.78	52 / 0.45	105 / 0.13	806 / 0.65
LO	103 / 0.21	38 / 0.04	8 / 0.07	260 / 0.33	34 / 0.02
RO	72 / 0.15	58 / 0.06	13 / 0.11	165 / 0.23	122 / 0.10
WO/SO	6 / 0.01	20 / 0.02	0 / 0	10 / 0.01	99 / 0.08

[b] DH = Deciduous hardwood, J = Juniper, LO = Live oak, RO = Red oak, WO/SO = White oak/Shin oak.

Table 3: Age classes of Ashe juniper species for GCW habitat type, GCW nesting type and non GCW habitat type classes, Fort Hood, TX.

Age Class	GCW/non OW	GCW/OW	Nesting Sites	Non-GCW Habitat
10-20	170	93	190	54
21-30	112	70	170	24
31-40	156	60	112	38
41-50	64	24	83	6
51-60	103	17	69	22
61-70	29	5	46	3
71-80	39	14	39	3
81-90	16	6	29	1
91-100	28	3	15	2
101-110	9	4	9	1
111-120	13	3	13	0
121-130	3	0	12	0

Figure 1: Mortality centers determined by photo interpretation, located on Fort Hood, TX.

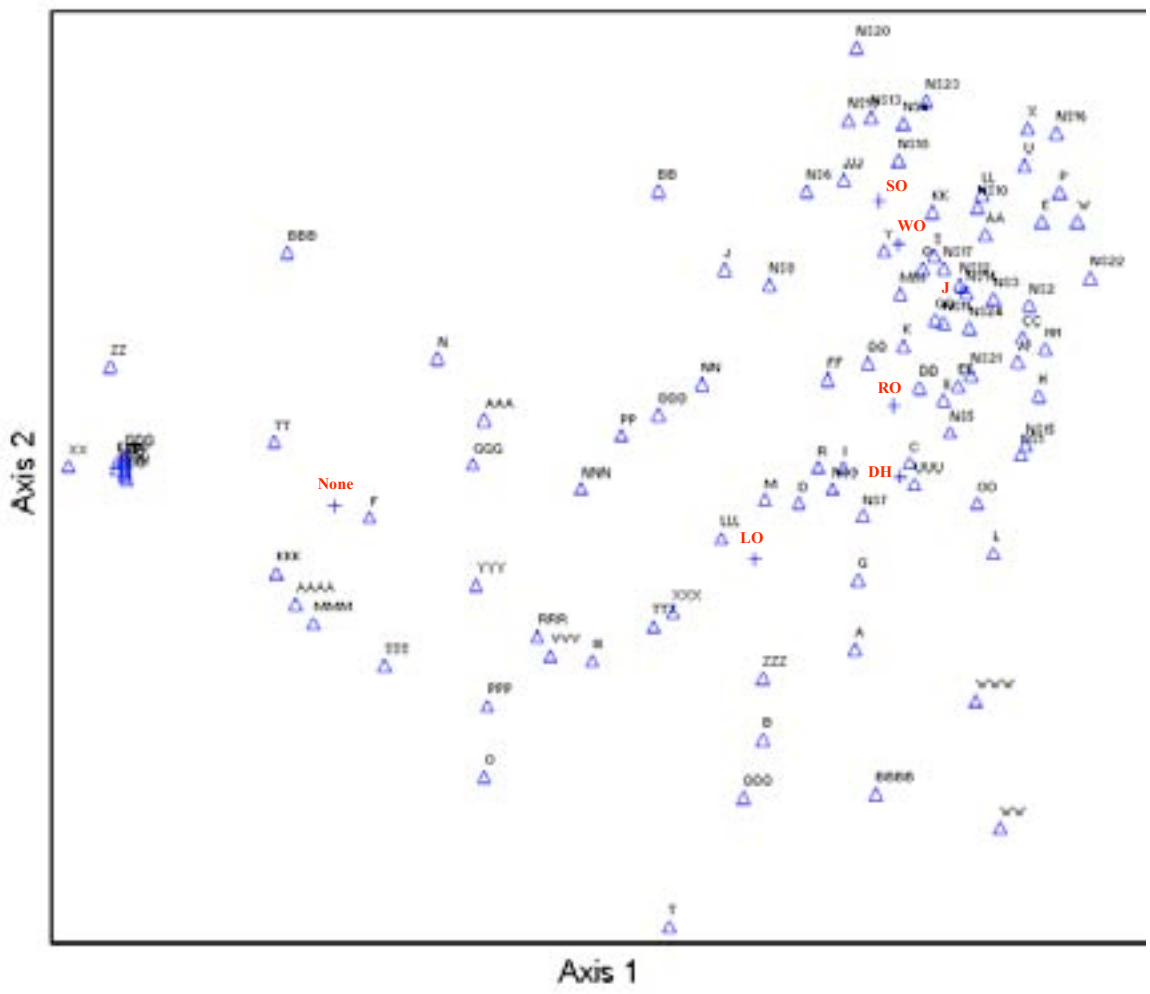

Figure 2: Non-metric multidimensional scaling (NMS) scatter plot to show the important species that are in golden cheeked warbler nesting sites, Fort Hood, TX.

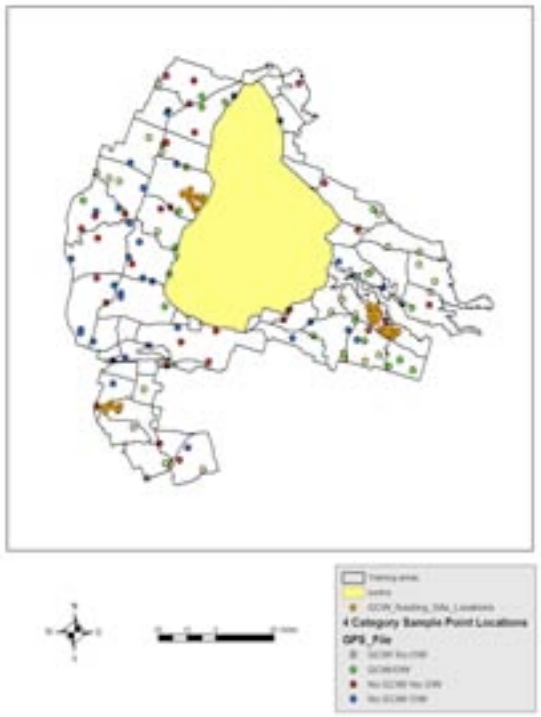

Figure 3: Locations of the five sampled plot categories located on Fort Hood, TX.

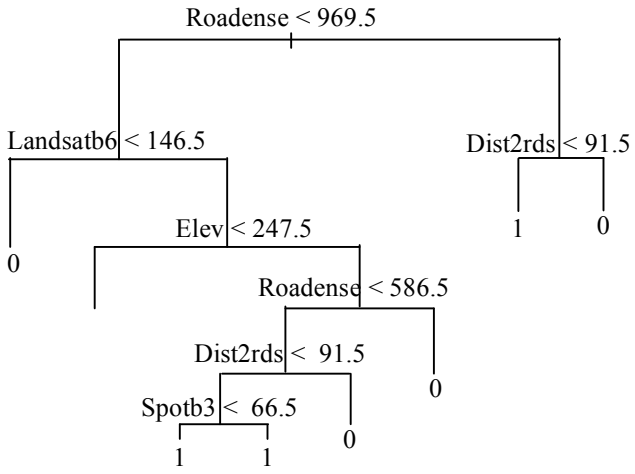

Figure 4: Classification tree model of GCW nesting site characteristics and map of predicted GCW nesting site habitat on Fort Hood, TX.

INSECT VECTORS OF THE OAK WILT FUNGUS IN MISSOURI AND TEXAS

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ABSTRACT

The oak wilt fungus, *Ceratocyctis fagacearum*, is transmitted overland by beetles in the family Nitidulidae and oak bark beetles in the genus *Psuedopityophthorus.* Studies were performed in 2005 and 2006 to determine the beetle species involved in transmission in Missouri and Texas. From this data, we hypothesize that *Colopterus truncatus*, *Co. niger* and *Co. semitectus* are vector species during the spring in Missouri and that the period of greatest risk for transmission is April and May with less risk in June. *Co. truncatus* was identified as a vector in Texas although other species may be involved. *Psuedopityophthorus pruinosis*, while present, could not be conclusively determined to be a vector in Texas. *Co. truncatus* appears to be a vector across the range of the disease while the involvement of other nitidulid beetle species varies with location. The contribution of oak bark beetles to the spread of the disease may also vary with location.

Key words: *Ceratocystis fagacearum*, *Colopterus*, Nitidulidae, *Pseudopityophthorus*

Beetles in the family Nitidulidae (Coleoptera) and oak bark beetles (Coleoptera: Curculionidae: Scolytinae) in the genus *Psuedopityophthorus* are considered the principal insect vectors of the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, in the U.S. (Gibbs and French 1980). Nitidulid beetles transmit the fungus when they visit fresh wounds on healthy oak (*Quercus* spp.) following visitation of fungal mats on oak wilt killed trees (Gibbs and French 1980). Oak bark beetles oviposit (lay eggs) in oak wilt-killed trees (Griswold and Bart 1954, Buchanan 1956). The larvae develop in the branches and when the new adults emerge they may carry the fungus on their bodies (Stambaugh et al. 1955, Rexrode, Kulman and Dorsey 1965, Berry and Bretz 1966). These adults can then transmit the disease when they feed on healthy oak trees (Buchanan 1958). Insect transmission, called above ground or overland transmission, can be prevented by removing wilted trees, avoiding the creation of wounds during periods of high risk, and painting unavoidable wounds made during this time period (French and Juzwik 1999, O'Brien et al. 2000, Juzwik et al. 2004).

Although the whole family of Nitidulidae has been implicated in transmission, research done in Minnesota suggests that only two species are the principal vectors in that area of the state (Juzwik, Skalbeck and Newman 2004b). In research done on nitidulid beetle transmission in Iowa, Norris (1956) concluded again that only a few species were likely to be important in transmission. Interestingly, while one key species (*Colopterus truncatus*) was the same between these two studies, the other species implicated in transmission were different. Also, while oak bark beetles are considered principal vectors in Missouri (Berry and Bretz 1966) and West Virginia (Rexrode and Jones 1971), research done in Minnesota (Ambourn, Juzwik and Eggers 2006) and Ohio (Rexrode 1967) does not support their role as principal vectors in the areas where the research was done. This leads to questions about which species are really involved in transmission and if the species involved are the same across the range of the oak wilt disease.

Studies were conducted in 2005 and 2006 in Missouri and Texas to determine the species responsible for transmission of the oak wilt fungus in each location. An effort was also made to identify periods of time during which there is a high risk of overland transmission. Information about the species involved and when they are capable of transmission can be used to find periods of high and low risk for transmission, thereby allowing for better disease management. Specifically, wounding studies were conducted in the spring of 2005 and 2006 in Missouri to collect nitidulid beetle species visiting fresh wounds on oaks in the red oak group (*Quercus* section *Lobatae*) and carrying the pathogen. One wounding study was also completed in Texas in February of 2005 by wounding red oak and live oak trees (*Quercus virginiana* and *Q. fusiformis*). In 2006, studies were done in Texas red oak wilt centers to trap for dispersing nitidulid beetles carrying the fungus in the late winter and spring. Also in Texas, trapping was done in the late winter and spring of 2005 and 2006 to collect dispersing oak bark beetles that may carry the fungus.

MATERIALS AND METHODS

Study Sites

All study sites in all years consisted of active oak wilt centers which were defined as areas with oak trees recently killed by oak wilt. Recently-killed red oaks producing mats served as sources of the fungus for wounding- and dispersing nitidulid beetle studies. These recently-killed oaks were also a possible source of collection for oak bark beetles.

Wounding and Beetle Collection

Wounds were created on healthy oaks to provide an infection court and a nitidulid beetle attractant. Each tree was wounded twice, once on the east-facing side and once on the west-facing side. A 5 cm hole saw was used to remove a round piece of bark to the outer xylem. The removed bark plug was re-inserted and held in place with a nail to create an attractive niche for the nitidulid beetles. Rain flaps were attached above the wound to prevent rain water from washing the insects out of the wounds below. Nitidulid beetles were collected from wounds once each day for six to nine days after wounding. Beetles were placed individually in sterilized 1.5 ml microcentrifuge tubes and the tubes were stored on ice during transport to the lab where they were stored at -2° C until they could be shipped on ice to the University of Minnesota where they were again stored at -2° C until processed.

Wounding studies were conducted using red oak trees in Missouri at two sites each year in 2005 and 2006 with a total of three sites, using one site both years. Wounding events occurred once a month in mid April, mid May and mid June. In Texas, there was one wounding event, using both red oak and live oak trees, at one site in late February of 2005.

Trapping of Dispersing Nitidulid Beetles

Dispersing nitidulid beetles were collected from four red oak sites in central Texas. At each site, wind-oriented funnel traps with fermenting flour dough and either *Colopterus truncatus* or *Carpophilus sayi* aggregation pheromone were placed in trees or bushes (Kyhl et al. 2002, Bartelt et. al 2004). Beetles were collected from traps and the baits were changed once a week for 14 weeks from early February through mid May, 2006. The contents of the traps were then shipped on ice to the University of Minnesota for processing. Beetles were placed individually in sterile micro centrifuge tubes and stored at -2° C until processed.

Trapping of Dispersing Oak Bark Beetles

Dispersing oak bark beetles were collected using window flight traps (without bait) from four red oak sites and four live oak sites in 2005 and from four red oak sites in 2006. Two traps were installed in the mid crown of each oak wilt killed tree on a rope and pulley system. Contents of the traps were collected once a week from early February to mid May. The contents of each trap were placed in a plastic bag and shipped on ice to the University of Minnesota for processing. Beetles were placed individually in sterile microcentrifuge tubes and stored at -2° C until processed.

Beetle Processing

All beetles were identified to species and the number of each species recorded. Beetles from all studies were assayed for pathogen presence following the same procedure. Beetles in their individual microcentrifuge tubes were macerated in 0.5 ml of sterile water with a tip sonicator to dislodge and expose fungal material. The macerated beetle in water was then used to create three ten-fold dilutions; 0.5 ml of suspension was then plated on each of three lactic acid amended potato dextrose agar plates. The plates were incubated in the dark at 24° C and examined after ten days for presence of the fungus (Cease and Juzwik 2001). The fungal colonies were identified by morphology and presence of endoconidia (Barnett 1953). The numbers of colonies on each plate for one selected dilution were counted and the colony-forming units per beetle were calculated.

RESULTS AND CONCLUSIONS

Missouri

Beetle Species Abundance. Fourteen species of nitidulid beetles were collected over the two years (Table 1). *Colopterus truncatus*, *Co. semitectus*, and *Co. niger* represented 64% of all beetles captured (Table 1). *Cryptarcha ampla* was the fourth most abundant species overall but still less than half as abundant as the third most abundant species (Table 1). Most beetles were collected from one of the three sites. This site, Little Lost Creek, was used in both years of the study. Fewer *Colopterus truncatus*, *Co. semitectus* and *Co. niger* individuals were captured at Little Lost Creek in June as compared to April and May (Fig. 1).

Beetle Species with *C. fagacearum*. Of 230 individual nitidulid beetles assayed, 23 yielded the oak wilt fungus. *Colopterus truncatus*, *Co. niger*, and *Co. semitectus* were the only beetle species contaminated with the fungus. Furthermore, only beetles captured in April and May yielded the pathogen (Fig. 1). A higher contamination frequency was found in 2006 with the majority of contaminated beetles captured in April (31% of all April beetles assayed) (Fig. 1).

Conclusions. We hypothesize that *Co. niger*, *Co. truncates*, and *Co. semitectus* (Fig. 2) are vectors of *Ceratocystis fagacearum* in east central Missouri based on their abundance in fresh wounds during spring months while contaminated with the pathogen. Ten percent of the nitidulid beetles captured that were assayed for the pathogen yielded the fungus. Although this shows that these nitidulid beetle species are capable of transmission, this is much lower than contamination rates of beetles from wounds in spring in Minnesota (Juzwik et al. 2004b) and in late February in Texas (Hayslett et al. 2005).

Contaminated beetles were found in greatest numbers in April with some in May and none in June. Current control measures for oak wilt in Missouri include avoidance of wounding from April through June to prevent infection. Our results suggest that April is a period of higher risk and that risk is lower in May and lowest in June. Additional data is needed to confirm this. If oaks could be wounded in June without risk of infection, this would give home owners, tree care professionals, and forest managers additional time to prune or harvest oaks.

Texas

Wound-inhabiting Nitidulid Beetles. One species, *Colopterus truncatus*, accounted for all (n=184) nitidulid beetles collected during late February from fresh wounds on oaks in the red oak and live oak stands at the Langford Ranch in 2005. High numbers of *Co. truncatus* collected from either red or live oaks yielded *C. fagacearum*. Overall, 83% of all collected beetles were carrying the fungus with contamination frequencies ranging from 71 to 100% depending on the tree species and wound age (Table 2).

Dispersing Nitidulid Beetles. Six species of nitidulid beetle were captured with the wind-oriented funnel traps, baited with *Colopterus* or *Carpophilus* pheromone and with dough; two *Colopterus* spp. (*Co. truncatus* and *Co. maculatus*), *Cryptarcha concinna*, and three *Carpophilus* spp. (*Ca. freemani*, *Ca. mutilates*, and *Ca. marginellus*). *Colopterus truncatus* accounted for 47% of nitidulid beetle counts at the Langford site and 6% at the Solana site (Table 3). *Carpophilus* spp. comprised 49% of nitidulid beetle counts at the Langford site and 74% of those

at the Solana site (Table 3). Peaks in beetle abundance over time were difficult to distinguish with the low beetle counts. Of 110 nitidulid beetles processed from this study, only three yielded *C. fagacearum* in culture; these were *Co. truncatus* collected from Langford Ranch between 7 - 14 March and 27 March - 4 April.

Dispersing Oak Bark Beetles. Few oak bark beetles were captured in the non-baited, window flight traps. Only two *Psuedopityophthorus pruinosis* individuals were collected from two traps in one tree out of the 24 traps placed in 12 live oak trees at three sites between 1 March and 15 May, 2005. Larger numbers of *P. pruinosis* were captured in traps located in red oaks. Over the same time period, 16 oak bark beetles were obtained from 5 trees of the 24 traps placed in 12 trees at 4 sites; similarly, 23 beetles were captured from 6 trees out of the 22 traps placed in 11 trees at 4 sites between 6 February and 12 May, 2006 (Fig. 3). Peaks in beetle abundance by month could not be determined from the low beetle counts. Of 36 *P. pruinosis* assayed, none yielded *C. fagacearum* in culture.

Conclusions. The abundance of *Co. truncatus* contaminated with *C. fagacearum* in fresh oak wounds is evidence of its role as vector of the oak wilt fungus in central Texas. Although this data was collected only for February in one site and one year, *Co. truncatus* have been collected in other studies in Texas. In our dispersing nitidulid beetle study, we collected *Co. truncatus* from the same site the next year and a few individuals carried the fungus. Dispersing *Co. truncatus* were collected from other sites in 1984, 1985, and 2006, although relatively few were captured and none carried the fungus (Appel et al. 1986, 1990). None of the other species collected in our study in 2006 carried the fungus. In 1984 and 1985, a few individuals from two species, *Colopterus maculatus* and *Cryptarcha concinna*, collected dispersing in oak wilt centers, were contaminated with *C. fagacearu*m (Appel et al. 1990). However, it is unknown if these species also visit fresh oak wounds. Based on this data, we suggest that *Co. truncatus* is a vector of *C. fagacearum* in central Texas and that other species are likely involved as well.

In 2006, only a few dispersing nitidulid beetles (0-15) were collected each week and so determination of any time when beetles are most active is difficult. However, the results of this study are consistent with those of Appel and colleagues (1990), except for the addition of late February (Texas Forest Service), for time periods during which nitidulid beetles are active and carry the oak wilt fungus (March through July).

As compared to a previous study done in Minnesota (Ambourn et al. 2006) using similar methods, oak bark beetle captures were overall very low. Although *P. pruinosis* was present in oak wilt centers and there was some evidence of colonization in oak wilt-killed red oaks, dispersing beetle numbers were so low that it seems unlikely that this species is a common vector of the fungus in central Texas. In terms of disease management, this data supports the current guidelines of avoiding wounds in late winter and spring to prevent spread by nitidulid beetles.

DISCUSSION

Colopterus truncatus was identified as a vector of the oak wilt fungus in both Missouri and Texas. This is in accordance with previous vector studies in Minnesota (Cease and Juzwik 2001, Juzwik et al. 2004b) and Iowa (Norris 1956) where it is also considered a vector based on mat surveys and wounding studies. Indeed, *Co. truncatus* is present throughout the United States (Parsons 1943) and has been identified either as a vector or as a possible vector in every location where nitidulid beetle transmission has been investigated. This species is found throughout the

central U.S. in a close association with the oak wilt disease cycle. Specifically, *Co. truncatus* is found both in fresh oak tree wounds (Dorsey and Leach 1956, Norris 1956, McMullen, Shenefelt and Kunts 1960, Juzwik, Skalbeck and Neuman 2004, Hayslett et al. 2005, Hayslett, Juzwik and Moltzan 2006) and on fungal mats (Curl 1955, McMullen et al. 1955, Norris 1956, Stambaugh and Fergus 1956, Cease and Juzwik 2001) where it is known to feed, oviposit and rear broods (Dorsey and Leach 1956, Norris 1956).

In a related Minnesota study, more *Co. truncatus* were found dispersing in active oak wilt centers than in oak stands without an active oak wilt center (Ambourn, Juzwik and Moon 2005). In an Iowa study, the timing of visitation by *Co. truncatus* adults to oak wilt fungal mats was viewed as favorable both for acquiring the fungus and for successful development of eggs deposited in the mats (Norris 1956). Specifically, *Co. truncatus* was found to visit mats very early when ascospores are sticky and viability is highest. These data support the theory of an ecological relationship between the insect and *C. fagacearum*.

The *Colopteru*s species *Co. niger* and *Co. semitectus*, identified by this study as vectors in east central Missouri, have been recognized as potential vectors by studies done in a few other states (Craighead, Morris and Nelson 1953, Curl 1955, Norris 1956, True et al. 1960). Interestingly, *Carpophilus sayi* and *Epuraea* were not indicated as possible vectors in Missouri or Texas but have been considered possible vectors in studies done in other states (Craighead, Morris and Nelson 1953, Curl 1955, Norris 1956, Stambaugh and Fergus 1956, McMullen, Shenefelt and Kuntz 1960, Juzwik, Skalbeck and Newman 2004). These data point to the involvement of only a few species in transmission of the fungus and that the suite of species may vary by location.

Research done in Missouri (Rexrode and Jones 1972), Ohio (Rexrode 1969), and West Virginia (Rexrode and Frame 1973) has provided evidence that two species of oak bark beetle, *Psuedopityophthorus minutissimus* and *P. pruinosis*, are capable of transmitting the oak wilt fungus. *P. minutissimus* is considered a vector in Missouri based on the high numbers of beetles emerging from oak wilt-killed trees and the high frequency with which they are contaminated with the oak wilt fungus (Berry and Bretz 1966). Although *P. minutissimus* has been implicated as a vector in Minnesota and Ohio, it does not appear to be a common vector in these locations. In Ohio, only 11% of oak wilt-killed trees were found to be colonized by *P. minutissimus* or *P. pruinosis* (Rexrode 1967). In Minnesota, only 0.4 to 1.3% of several hundred *P. minutissimus* individuals dispersing in oak wilt-killed tree crowns were found to carry the pathogen (Ambourn, Juzwik and Eggers 2006).

Psuedopityophthorus pruinosis has been implicated as a vector in West Virginia, again based on high contamination frequencies (Rexrode and Jones 1971). In our Texas study, only a few *P. pruinosis* individuals were found dispersing in the crowns of the oak wilt-killed trees examined. This suggests that *P. pruinosis* is infrequently involved in transmission of the oak wilt fungus in central Texas. Again, this data indicates that the common insect vector species may vary with location.

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Table 1. Nitidulid beetle captures by species from fresh wounds on red oaks in spring at three sites in Missouri.

	Number of Beetles				
	Perry 2005	**Lost Creek 2005**	**Prairie 2006**	**Lost Creek 2006**	**All sites**
Colopterus truncatus	0	4	4	66	74
Colopterus semitectus	0	11	5	48	64
Colopterus niger	0	17	3	24	44
Colopterus maculatus	1	9	1	1	12
Carpophilus sayi	1	1	0	19	21
Carpophilus corticinus	0	1	1	8	10
Cryptarcha ampla	5	14	0	0	19
Cryptarcha concinna	0	7	1	0	8
Lobiopa Undulata	0	14	2	1	17
Glischerochilus obtusus	0	5	0	2	7
Aphicrosis ciliatus	0	5	0	0	5
Prometopia sexmaculata	0	3	0	1	4
Epurea spp.	0	0	0	1	1
Total	7	91	17	171	286

Table 2. Numbers of *Colopterus truncatus* collected from fresh wounds on 13 healthy red and 10 healthy live oak trees near an oak wilt center at a ranch in central Texas in February 2005 and assayed for the oak wilt fungus.

Tree stand type	**Days after wounding**	**Number of beetles**	
		Assayed	**with Cf [a]**
Live oak	1	-- [b]	--
	2	43	30
	3	0	0
	4	0	0
	5	0	0
	6	5	4
Red oak	1	109	97
	2	6	6
	3	2	2
	4	0	0
	5	17	12
	6	2	2

[a] Number of beetles contaminated with *Ceratocystis fagacearum*.
[b] Not applicable, no collections were made in live oak on day one.

Table 3. Nitidulid beetle captures by location collected with baited funnel traps placed in red oak wilt centers at four central Texas locations during late winter and spring, 2006.

	Number of beetles captured at:			
Species	**Langford**	**Solana**	**Johnson**	**TNLA**
Colopterus truncatus	27	3	0	2
Colopterus maculatus	0	4	4	3
Cryptarcha concinnus	2	7	0	2
Carpophilus spp.	28	40	0	6

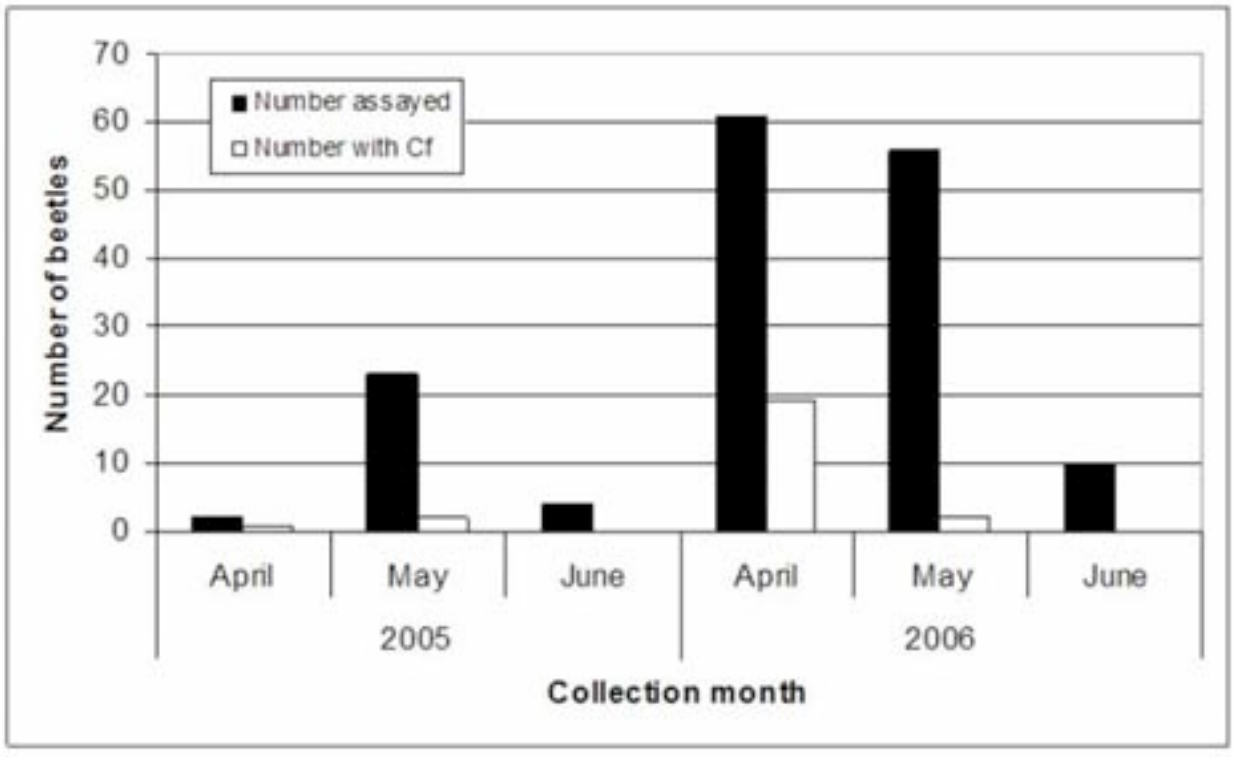

Figure 1. Number collected and frequency of *Ceratocystis fagacearum* (Cf) isolated from three *Colopterus* spp. (*Co. truncatus*, *Co. niger*, and *Co. semitectus*) collected from fresh wounds on 12 healthy red oak trees at Little Lost Creek Conservation Area in Missouri.

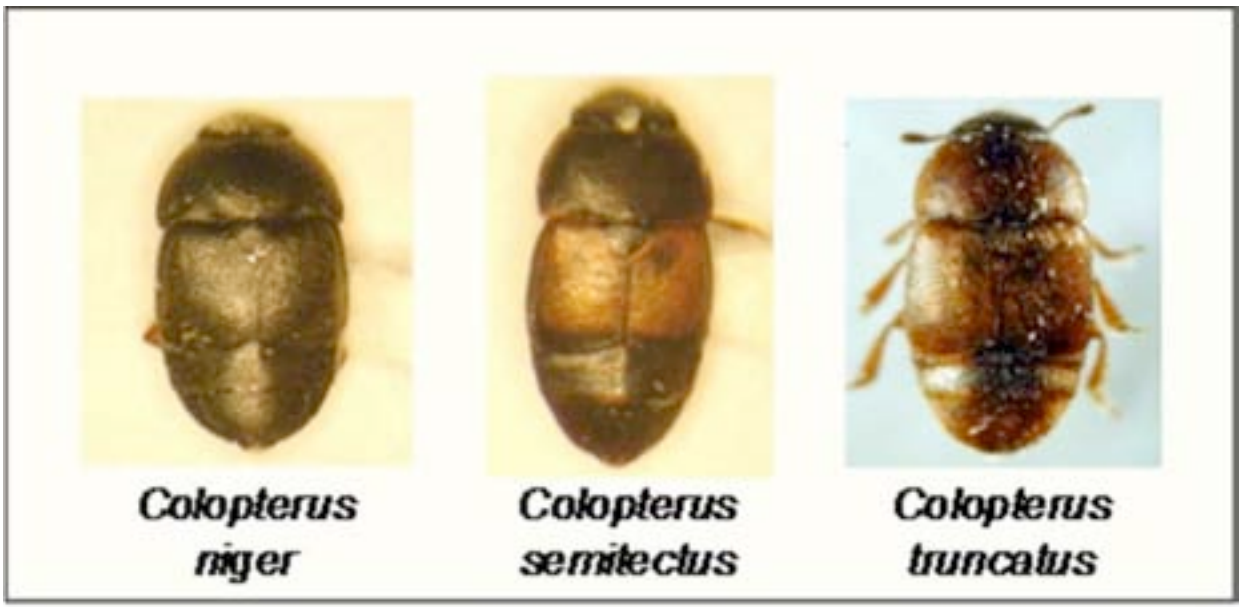

Figure 2. Photographs of the three *Colopterus* spp. identified as vectors of the oak wilt fungus in east central Missouri. Photographs taken by Maya Hayslett and Angie Ambourn.

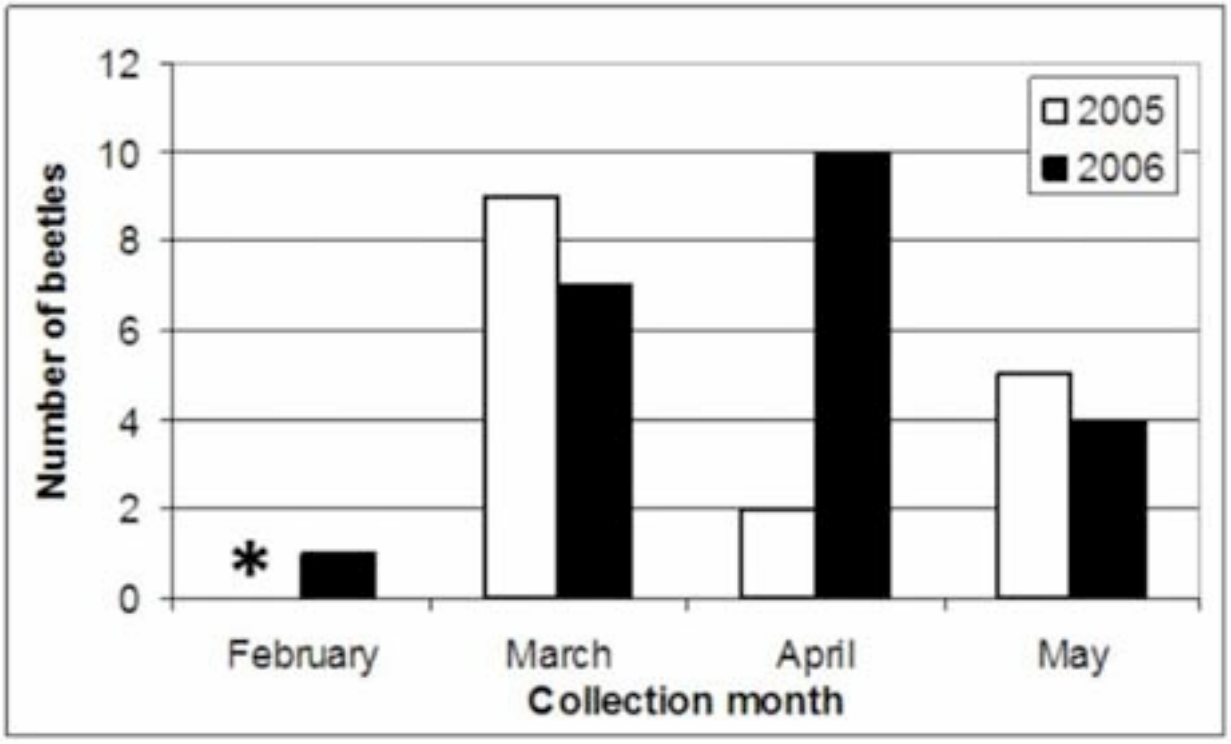

Figure 3. Number of *Psuedopityophthorus pruinosis* collected using non-baited flight traps in the crowns of oak wilt killed red oaks in central Texas. *No collections were made in February, 2005.

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 nititulids 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 -97º45'12"W and 30º28'23"N. The tree species on the ranch consisted mainly of live oaks (*Q. fusiformis*), but cedar elm (*Ulmus crassifolia*) and gum bumelia (*Bumelia lanuginose)* 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 x 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 x 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 poptools (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 0.4-049, 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^{o} 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 then 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 0.2-0.29	0.3-0.39	0.4-0.49	0.5-0.59	Mean	Standard Error
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, $P = 0.10$.

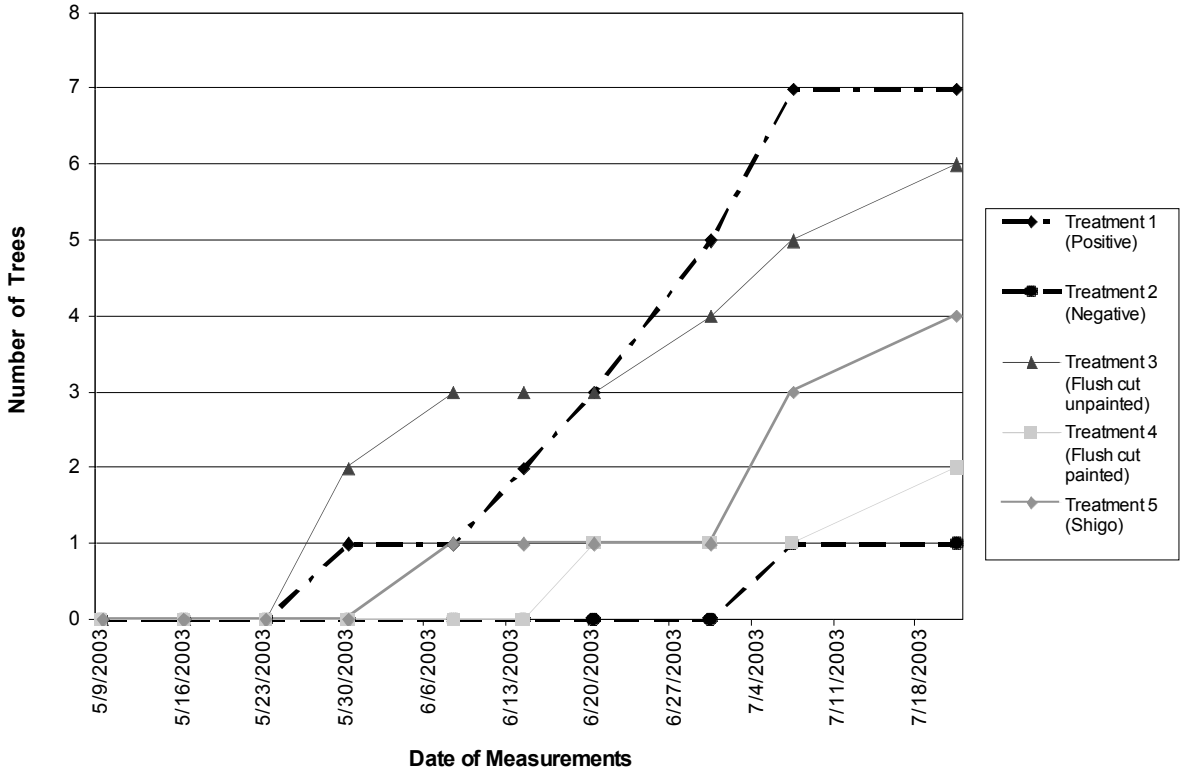

Figure 1. Symptom expression of *Quercus fusiformis* after inoculation with *Ceratocystis fagacearum* on April 30, 2003.

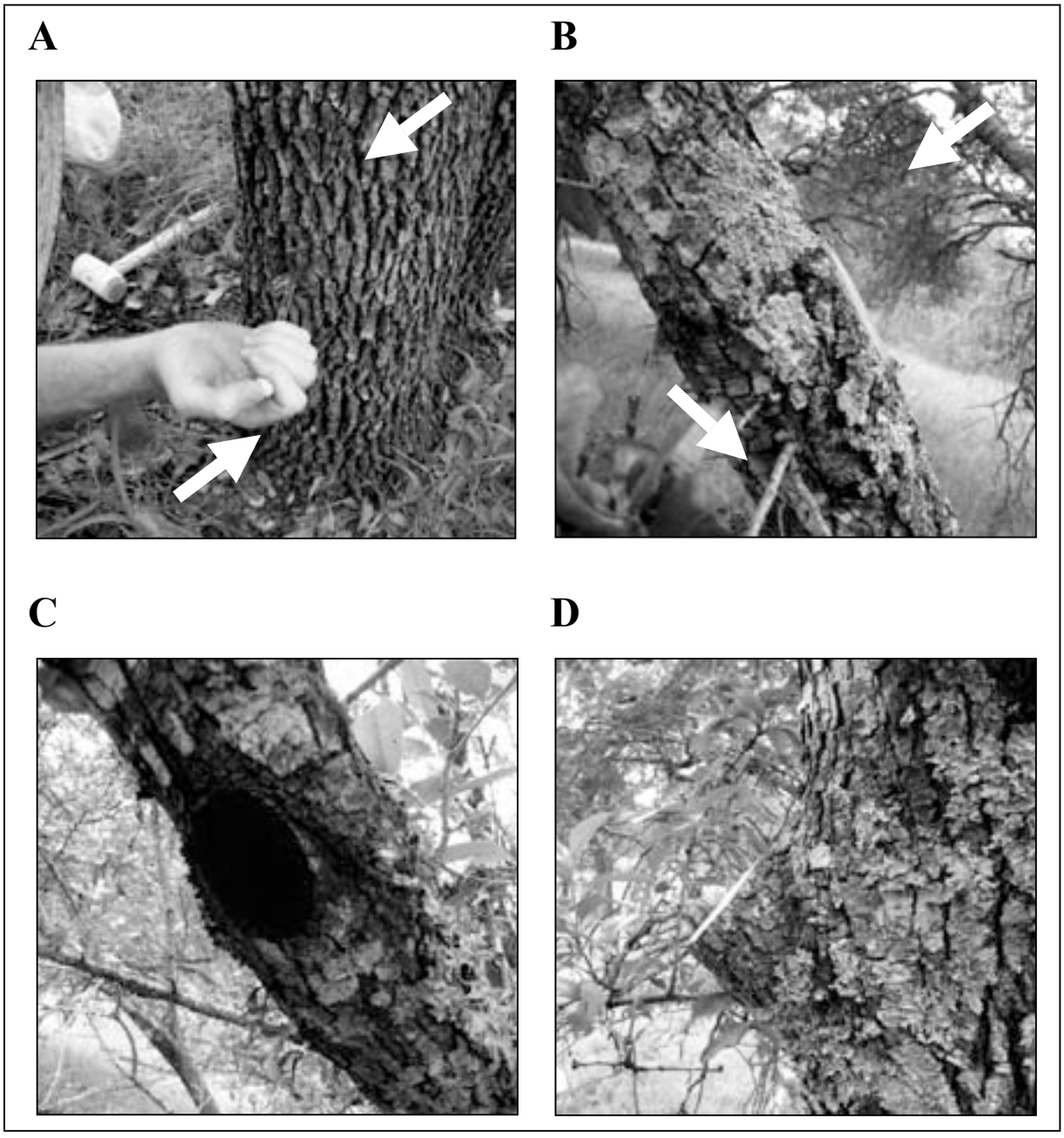

Figure 2. Treatment types for inoculation of *Q. fusiformis* with *C. fagacearum*. A = positive and negative (Treatments I and II), B = flush pruning cut, unpainted (Treatment III), C = flush pruning cut, painted (Treatment IV) and D = proper pruning cut (Treatment V). Arrows indicate location of wounds.

A Laboratory-Induced Hypovirulent Strain of the Oak Wilt Fungus

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ABSTRACT

A strain of *Ceratocystis fagacearum* with debilitated respiration, as measured by an increase in alternative oxidase activity, was selected in the laboratory after exposure to ethidium bromide and ultra-violet light mutagenesis. The mutant, PM447, showed altered morphology, increased levels of alternative oxidase activity, and reduced virulence when compared to the wild type strain. PM447 protected 28-day-old seedlings from oak wilt disease when seedlings were inoculated two-weeks prior to inoculation by virulent strains. In an effort to apply this technology to a wider application, we expanded the parameters of the original study to include challenges beyond two weeks, varying the strain of virulent wild-type used in the challenge inoculation, varying the number of spores of PM447, and using the mutant to protect saplings and mature trees in field plots. In general, PM447 has been shown to protect challenged seedlings and delay disease onset in mature trees; however, its efficacy as a biocontrol agent in the field is poor and will require a better understanding of the mechanisms of protection.

Key words: Biocontrol agent, *Ceratocystis fagacearum*, hypovirulence

The term 'hypovirulence' was first used to describe strains of the chestnut blight pathogen, *Cryphonectria parasitica* (Murrill) Barr, that were decreased in virulence (Grente 1965). These strains were recovered from non-lethal, healing cankers on chestnut trees and typically displayed abnormal growth and morphology in culture. It was later determined that the hypovirulent phenotype was due to the presence of cytoplasmic double-stranded RNA within the fungal mycelium (Day et al. 1977). Some hypovirulent strains of *C. parasitica* were found that lacked dsRNA hypoviruses (Fulbright 1985, Baidyaroy et al. 2000). Mahanti et al. (1993) established that these isolates had increased levels of alternative oxidase activity, indicative of mitochondrial dysfunction. Additionally, the hypovirulent phenotype was transmissible via hyphal anastomosis and maternally inherited in crosses, suggesting the role of mitochondrial mutations in hypovirulence in these isolates.

Shaw (1999) attempted to duplicate mitochondrial-based hypovirulence in *Ceratocystis fagacearum* (Bretz) Hunt, the fungal pathogen that causes oak wilt. Conidia from a wild-type strain, "Fenn", were exposed to ethidium bromide and UV light and then screened for slow growth, an indicator of possible mitochondrial dysfunction. The mitochondrial origin of the phenotype was determined by testing for alternative respiration (cyanide resistance and salicylhydroxamic acid sensitivity) and maternal inheritance of the trait. One mutant, PM447, appeared to satisfy the above requirements and was subsequently used in seedling assays similar to that developed by Fenn, Durbin and Koontz (1975). Using 28-day-old seedlings maintained in a growth chamber, Shaw found that seedlings first inoculated with the hypovirulent strain PM447 and then challenged with the wild-type Fenn two weeks later, displayed significantly less symptom development when compared to seedlings inoculated with Fenn only or those seedlings

challenged at 0 or 1 weeks. In preliminary efforts to apply this technology to a wider application, we repeated Shaw's earlier work, expanded the parameters to include challenges beyond two weeks, varied the strain of wild-type used in the challenge inoculation, varied the spore load of PM447, and included mature trees from several field plots in these studies.

MATERIALS AND METHODS

Spores from PM447 cultures stored for two years at 4-6ºC were screened for slow growth, and conidia from the slowest growing of these were collected. These conidia were then plated and screened for slow growth following germination. A spore suspension (10^5/ml) was made by combining conidia with water and 20% glycerol. The resulting suspension was stored at -80ºC and used for all subsequent PM447 inoculations. Wild-type isolates used in the studies were originally obtained from diseased trees (Westcott and Beal) or from cultures stored in collections.

Single-spore cultures of all isolates used were plated on potato-dextrose agar (PDA) and allowed to grow for 14 days at room temperature. Conidia were collected by pipetting 2 ml of distilled water onto the plates and rubbing the top of the mycelia with a glass rod. The resulting suspension was strained through Miracloth™ and the conidial density was adjusted to $1x10^5$ conidia/ml with distilled water and glycerol to make a 20% glycerol solution. Suspensions were divided into 1ml aliquots and maintained at -80ºC.

Greenhouse and Growth Chamber Experiments

Experiments performed in the greenhouse and growth chamber utilized 28- to 35-day-old red oak (*Quercus rubra*) seedlings; red oak seedlings were used in the greenhouse study, and pin oak (*Q. palustris*) was used for the growth chamber study (*Q. rubra* seed unavailable). Stratified seed were planted into 16 ounce cups containing Baccto™ planting mix. The growth chamber was maintained at 26º C with a 16-hour daylight period.

For inoculations, a 10 ul drop of conidial suspension was placed at the base of the stem approximately 2 cm above the soil line. A 26-gauge needle was then inserted through the droplet into the stem at a 45-degree angle. Absorption of the droplet was observed, indicating successful uptake of the suspension into the xylem. Inoculated seedlings were monitored weekly for symptom development.

The disease rating of seedlings was based on the degree of symptom expression at six weeks (greenhouse) or eight weeks (growth chamber) post-wild-type inoculation using a 0 to 5 scale (Table 1).

Field Plot Experiments

All trees used in the Beaumont/East Farm study were red oak and trees at the Jackson site appeared to be a mix of red and northern pin oak (*Q. ellipsoidalis*). The Beaumont/East Farm study had three tree category types based on their diameter; type 1 trees were saplings with a diameter at breast height (d.b.h.) of 2-3 cm, type 2 trees had a d.b.h. equal to 5-6 cm, and type 3 trees were mature trees with a d.b.h. greater than 12 cm. All other trees used in the field plots were considered type 3. Inoculation wounds were made at 1.4 meters above ground into the north side of the trunk by drilling a small hole into the xylem. In cases where trees received more than one inoculation, subsequent inoculation sites were located 1/4 of the way around the trunk to the left of the previous inoculation location. Type 1 trees received two doses of 10ul inoculum, five minutes apart, for a total of 20 ul. Type 2 trees received 50 ul inoculum and type

3 trees received 1 ml inoculum. Trees were rated as healthy (0), intermediate (1), or wilted (2). Intermediate ratings were assigned to trees that developed wilt symptoms that did not progress beyond 60% crown wilt over a 1- to 2-year period. A disease rating of 2 was given to trees with advanced stages of wilt that did not recover by the following year.

Statistical analysis of the data was performed using the Genmod procedure with SAS v.9.1 software. In some cases when the model fit using Genmod was in question, the Glimmix procedure was utilized. A p value less than or equal to 0.05 was used to determine statistical significance of the variable/parameter in question.

RESULTS

Greenhouse Inoculation Studies

The greenhouse study was designed to examine how the timing between inoculation with PM447 and wild-type challenge inoculations, as well as varying the wild-type strains (Fenn, Westcott, or Beal), affect symptom development in seedlings. Disease ratings were significantly higher in rep 2, so the data from both experiments were analyzed separately. There was no significant difference between challenges at 0 weeks and seedlings inoculated with the corresponding wild-type strain only (Table 2 and Fig. 1). Disease ratings of seedlings challenged at either 1, 3, or 4 weeks, however, were significantly lower than those of seedlings inoculated with a wild-type strain only or those co-inoculated with PM447 and a wild-type. There was no significant difference between disease ratings for 3 and 4 week (rep 1) or 1 and 3 week (rep 2) challenges for any of the isolates.

Growth Chamber Inoculation Studies

Growth chamber treatments included a range of spore concentrations of PM447 to determine the effect on disease progression in seedlings that were challenged two weeks later with a wild-type strain, Westcott. There were no significant differences in disease ratings among seedlings inoculated with different concentrations of PM447 ($p=0.6$) (Table 3 and Fig. 2). Seedlings that were challenged with Westcott had significantly higher disease ratings than those only inoculated with PM447 ($p=.0004$). However, there was no significant difference in disease rating of challenged seedlings compared to seedlings only inoculated with Westcott ($p=0.1$).

East Farm/Beaumont Field Plot

Treatments within the Beaumont/East Farm study were set up to determine the effect of timing between initial inoculation with PM447 and challenge inoculations with a wild-type strain (Westcott), on disease development on older trees (Tables 4 and 5). All control trees remained symptomless. There was no variation in disease ratings for any type 1 tree regardless of treatment (not including controls); all trees inoculated with either PM447 or Westcott wilted within the first year and did not leaf out the following year. Ratings for tree type 2 trees varied, showing a trend similar to that observed with tree type 3 trees; however, there were no significant differences in treatments. There was a significant effect of the timing of the challenge inoculation on disease rating with type 3 trees ($p=.002$) one year after inoculation. Symptom development on 2- and 3-week-challenged trees progressed much slower than on trees challenged at 1 week or inoculated with the wild-type only. However, all trees eventually wilted completely in subsequent years.

Jackson Field Plot

The Jackson study was designed to assess how varying the spore load of PM447 affects symptom expression when PM447 is inoculated alone. These treatments were done to determine if lower spore loads may reduce symptoms so as to provide protection rather than killing the tree. Conidial concentrations of 10^1, 10^2, 10^3, and 10^1+10^1 did not produce symptoms that were significantly different from each other or the water controls (Fig. 4). Inoculation with 10^5, 10^2+10^2, 10^3+10^3, $10^1+10^1+10^1$, or $10^2+10^2+10^2$ spores produced symptoms that were not significantly different from each other or the Westcott (wild-type) control (Fig. 3). The former treatments (including the water controls) produced significantly lower disease ratings than the latter group of treatments (including the wild-type control) ($p=0.01$) (Table 6 and Fig. 3).

Beaumont Field Plot

The Beaumont study focused on the consequences of varying the spore load of PM447 in combination with two-week-challenge inoculations with the virulent Westcott strain on disease development (Table 7). All water-inoculated (control) trees remained symptomless. There was no significant difference between ratings for those trees inoculated with Westcott only and those trees first inoculated with PM447 and then challenged with Westcott 2 weeks later ($p=0.9$). Ratings for trees inoculated with PM447 only were significantly less than for other treatments ($p=0.02$); however, there was no significant effect in regard to the spore concentrations used in the study.

DISCUSSION

Results from the greenhouse experiments are similar to those reported earlier by Shaw (1999); that is, PM447 was reduced in its ability to cause severe symptoms when inoculated into young seedlings alone, and PM447 provided protection to seedlings subsequently challenged by wild type strains several days later. However, Shaw observed protection only when PM447 was inoculated 2 weeks prior to the wild type challenge inoculation and in this study we observed protection regardless of timing.

Growth chamber results again demonstrated the hypovirulent nature of PM447, but in this study were not as promising as a biocontrol agent. Despite lower disease ratings for challenged seedlings compared to wild type Westcott isolate alone, statistically there was no significant effect of PM447 when inoculated two weeks prior to inoculation with a wild-type strain. Although different oak species were used in these studies, all species of red oaks are thought to have similar susceptibilities. The observed disparity could be due to the smaller sample size used in the growth chamber study coupled with the highly variable seedling response, resulting in a statistically insignificant outcome. It should be noted that Shaw observed significant and reproducible biocontrol outcomes with PM447 in growth chamber studies with red oak (Shaw 1999).

Field plot results varied, and although PM447 appeared to delay symptom development in mature trees, in general it was not an effective biocontrol as trees generally wilted completely within 1-2 years. At the Jackson site, trees inoculated with 10^1, 10^2, 10^3, or 10^1+10^1 (10^1 followed two weeks later by 10^1) spores of PM447 experienced significantly less symptom development than trees inoculated at other (higher) concentrations. These results show that PM447, at relatively low concentrations may not ultimately induce wilt as had previously been

observed using 10^5 spores. This would be promising for its potential role as a biological control agent; however, results at the Beaumont plot indicated that these lower PM447 inoculum loads did not affect the development of wilt upon challenge inoculation with a wild-type strain. While the average disease rating for trees in the "Challenge: 10^1+10^3" treatment in the Beaumont plot appears to be lower than that observed with the other challenge treatments, statistically, there was no significant difference between challenge treatments and inoculation with the wild-type strain, Westcott, only. This lack of significance may be due to the relatively small sample size per treatment and the overlapping range of disease ratings for each treatment.

In general, PM447 appeared to delay symptom development in subsequently challenged seedlings and trees; however, its efficacy as a biocontrol agent is not well understood. The results indicate that red oaks do respond to the presence of PM447 in some capacity that slows the progression of the disease. Perhaps the slow growth of PM447 in culture translates to slower growth and/or reduced fitness within a seedling or tree, thus enabling the host to respond to the presence of this pathogen. It is likely that this response is an induced defensive reaction that restricts the pathogen to some extent, although the pathogen is ultimately able to overcome any defenses produced by the host. Based on these studies, materials capable of inducing an acquired resistance response in oak should be tested for their efficacy to oak wilt.

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Table 1. Disease ratings (DR) for seedlings based on level of symptom expression. Statistical analysis of greenhouse trial results utilized the DR recorded at 6 weeks post-wild-type inoculation. For the growth chamber study, the DR at 8 weeks was evaluated.

Disease rating	Symptom expression
0	Symptomless
1	Leaves with mild bronzing of tips; less than 25% leaf area affected
2	Mild curling and drying of leaves with bronzing more apparent; up to 50% leaf area affected
3	Leaves curled and dry with bronzing of up to 75% of leaf area
4	Leaves severely curled and dry with bronzing nearly to petiole
5	Leaves entirely brown (though few scattered green flecks may remain); defoliation common; nearly 100% of leaf area affected

Table 2. List of treatments assessed in the greenhouse study to evaluate the effects of wild-type strain used and timing between PM447 and challenge inoculations on symptom development.

Treatment	Description
Control	Seedlings inoculated with water
PM447 only	Seedlings inoculated with the hypovirulent, PM447 strain
Wild-type only	Seedlings inoculated with one wild-type strain (Fenn, Westcott, or Beal)
Challenge: 0 weeks	Seedlings co-inoculated with PM447 and one wild-type strain (Fenn, Westcott, or Beal)
Challenge: 1 week	Seedlings inoculated with PM447, then challenge inoculated 1 week later with a wild-type strain (Fenn, Westcott, or Beal)
Challenge: 3 weeks	Seedlings inoculated with PM447, then challenged 3 weeks later with a wild-type strain (Fenn, Westcott, or Beal)
Challenge: 4 weeks	Seedlings inoculated with PM447, then challenged 4 weeks later with a wild-type strain (Fenn, Westcott, or Beal)

Table 3. List of treatments included in the growth chamber experiment to determine the effect of PM447 spore load on wilt development in seedlings challenged with a wild-type strain two weeks later.

Treatment	Description
Control	Seedlings inoculated with water
PM447: 10^3	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447
PM447: 10^4	Seedlings inoculated with 10ul of 10^4 spores/ml of PM447
PM447: 10^5	Seedlings inoculated with 10ul of 10^5 spores/ml of PM447
PM447: 10^3+10^3	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447, then were inoculated with 10ul of 10^3 spores/ml of PM447 two weeks later
Westcott	Seedlings inoculated with the wild-type strain, Westcott
Challenge: 10^3	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447, then challenge inoculated two weeks later with Westcott
Challenge: 10^4	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447, then challenge inoculated two weeks later with Westcott
Challenge: 10^5	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447, then challenge inoculated two weeks later with Westcott
Challenge: 10^3+10^3	Seedlings inoculated with 10ul of 10^3 spores/ml of PM447, then were inoculated with 10ul of 10^3 spores/ml of PM447 two weeks later, then were challenged with Westcott two weeks after the second inoculation with PM447

Table 4. List of treatments used in the East Farm/Beaumont study to evaluate the effect of the timing between PM447 and challenge inoculations on disease development. Each treatment group included multiple trees of each size type: type 1 trees had a dbh=2-3 cm, type 2 trees had a dbh=5-6 cm, and type 3 trees had diameters greater than 12 cm.

Treatment	Description
Control	Trees were inoculated with water
PM447	Trees were inoculated with the hypovirulent strain, PM447
Westcott	Trees were inoculated with the wild-type strain, Westcott
Challenge: 1 week	Trees were inoculated with PM447, then challenge inoculated with Westcott one week later
Challenge: 2 weeks	Trees were inoculated with PM447, then challenge inoculated with Westcott two weeks later
Challenge: 3 weeks	Trees were inoculated with PM447, then challenge inoculated with Westcott three weeks later

Table 5. Average disease ratings (DR) of type 3 trees (dbh > 12 cm) within the East Farm/Beaumont plot one year (2005) and two years (2006) after inoculation. Trees were assigned a DR of 0 if they expressed no symptoms, a 1 if they had mild to moderate symptoms (less than 60% of crown affected), or a 2 if they developed advanced symptoms or completely wilted.

Treatment	Average DR: 2005	Average DR: 2006
Control	0	0
PM447	1	2
Westcott	2	2
Challenge: 1 week	2	2
Challenge: 2 weeks	1	2
Challenge: 3 weeks	1	2

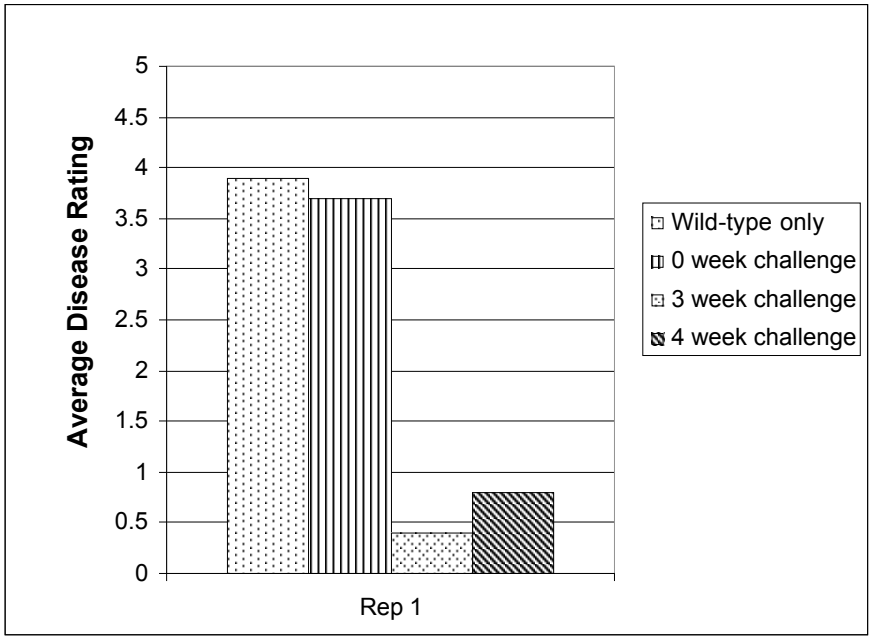
Table 6. List of treatments used at the Jackson site to ascertain the effect of PM447 spore load on symptom development in red oaks. The average disease ratings (DR) for trees within each treatment group are given. Trees were scored based on the level of symptom development one year following inoculation; a DR equal to 0 indicates trees developed no symptoms, a 1 was assigned to trees with mild to moderate symptoms (less than 60% of crown affected), and a 2 represented those trees displaying advanced or complete wilt.

Treatment	Description	Average DR*
Control	Trees were inoculated with water	0 a
Westcott	One tree was inoculated with the wild-type strain, Westcott	2 b
PM447: 10^1	Trees were inoculated with 10^1 spores of PM447	0 a
PM447: 10^2	Trees were inoculated with 10^2 spores of PM447	0 a
PM447: 10^3	Trees were inoculated with 10^3 spores of PM447	0.5 a
PM447: 10^5	Trees were inoculated with 10^5 spores of PM447	2 b
PM447: 10^1+10^1	Trees were inoculated with 10^1 spores of PM447, then were inoculated two weeks later with 10^1 spores of PM447	0.33 a
PM447: 10^2+10^2	Trees were inoculated with 10^2 spores of PM447, then were inoculated two weeks later with 10^2 spores of PM447	1 b
PM447: 10^3+10^3	Trees were inoculated with 10^3 spores of PM447, then were inoculated two weeks later with 10^3 spores of PM447	1 b
PM447: 10^1+10^1+10^1	Trees were inoculated with 10^1 spores of PM447, then were inoculated one week later with 10^1 spores of PM447 and again one week afterward with 10^1 spores of PM447	1.67 b
PM447: 10^2+10^2+10^2	Trees were inoculated with 10^2 spores of PM447, then were inoculated one week later with 10^2 spores of PM447 and again one week afterward with 10^2 spores of PM447	1.33 b

*Disease ratings for each treatment followed by the same letter are not significantly different at p=0.05.

Table 7. List of treatments tested in the Beaumont study to determine the effect of variable spore concentrations of the hypovirulent strain, PM447, on symptom development in trees challenged with a wild-type strain two weeks after inoculation with PM447. The average disease ratings (DR) for trees within each treatment are given. Disease ratings were recorded the year following inoculation and were assigned as follows: 0 = no apparent symptoms, 1 = mild to moderate symptoms (less than 60% of crown affected), and 2 = advanced or complete wilt. In general, trees inoculated with PM447 only had significantly lower disease ratings than trees inoculated or challenged with Westcott when treatments were combined. None of the challenge treatments were significantly different than the Westcott treatment.

Treatment	Description	Average DR
Control	Trees were inoculated with water	0
PM447: 10^1	Trees were inoculated with 10^1 spores of PM447	1
PM447: 10^3	Trees were inoculated with 10^3 spores of PM447	2
PM447: 10^5	Trees were inoculated with 10^5 spores of PM447	2
PM447: 10^1+10^1	Trees were inoculated with 10^1 spores of PM447, then inoculated a second time with 10^1 spores of PM447 two weeks later	0
PM447: 10^1+10^3	Trees were inoculated with 10^1 spores of PM447, then inoculated a second time with 10^3 spores of PM447 two weeks later	0.67
PM447: 10^3+10^3	Trees were inoculated with 10^3 spores of PM447, then inoculated a second time with 10^3 spores of PM447 two weeks later	0.67
Westcott	Trees were inoculated with the wild-type strain, Westcott	2
Challenge: 10^1	Trees were inoculated with 10^1 spores of PM447, then challenged two weeks later with Westcott	2
Challenge: 10^3	Trees were inoculated with 10^3 spores of PM447, then challenged two weeks later with Westcott	2
Challenge: 10^5	Trees were inoculated with 10^5 spores of PM447, then challenged two weeks later with Westcott	2
Challenge: 10^1+10^1	Trees were inoculated PM447: 10^1+10^1 as described above, then challenged with Westcott two weeks later	1.67
Challenge: 10^1+10^3	Trees were inoculated PM447: 10^1+10^3 as described above, then challenged with Westcott two weeks later	0.67
Challenge: 10^1+10^3	Trees were inoculated PM447: 10^3+10^3 as described above, then challenged with Westcott two weeks later	1.67



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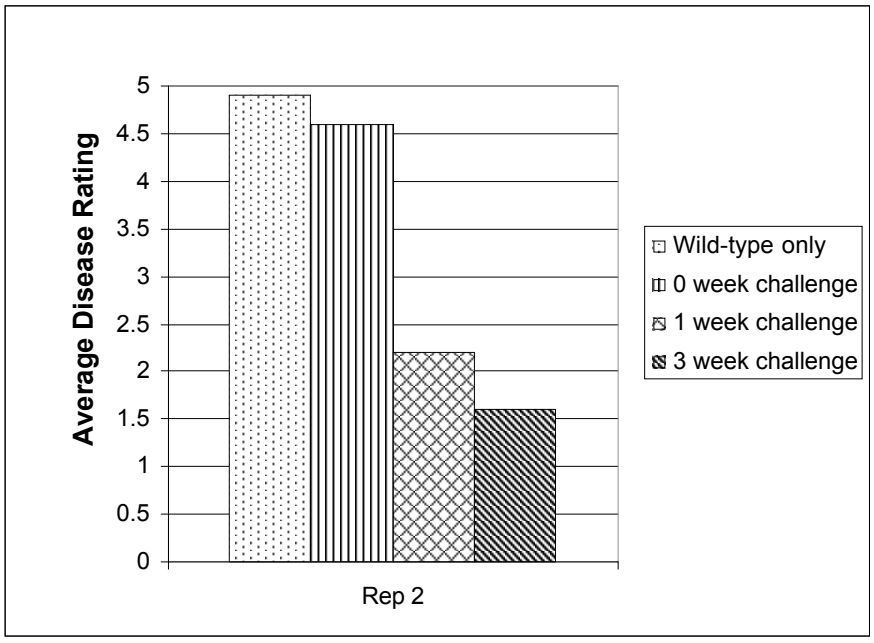

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Figure 1. Average disease ratings for red oak (*Quercus rubra*) seedlings six weeks after inoculation with a wild-type strain. Data from all wild-type strains utilized was combined as there was no significant strain effect (p=0.9). Challenged seedlings were first inoculated with the hypovirulent strain, PM447, and then challenge inoculated with a wild-type strain at A) 0, 3, or 4 weeks (rep 1) or B) 0, 1, or 3 weeks (rep 2) after PM447 inoculation. Seedlings challenged at 3 and 4 weeks (rep 1) or 1 and 3 weeks (rep 2) had significantly lower disease ratings than seedlings inoculated with a wild-type strain only or challenged at 1 week.

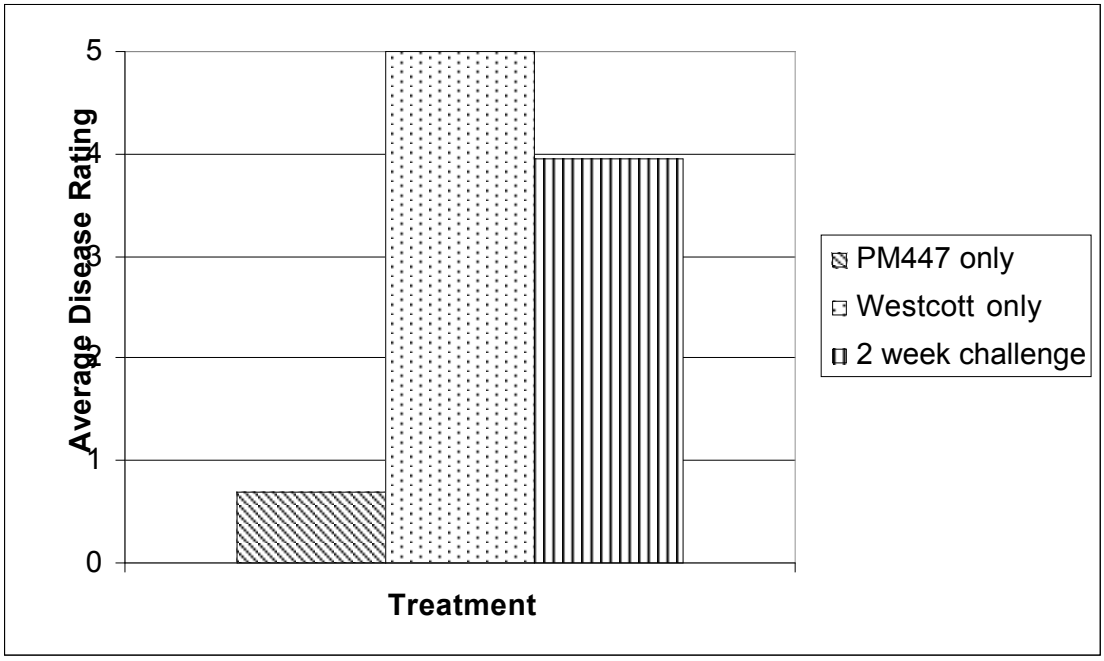

Figure 2. Average disease ratings for pin oak (*Quercus palustris*) seedlings eight weeks after final inoculations. Seedlings were inoculated with either the hypovirulent strain PM447 only, the wild-type strain, Westcott, only, or PM447 followed by a challenge inoculation with Westcott 2 weeks later. All seedlings inoculated with PM447 received one of four different conidial concentrations; however, no spore load effect was observed for either PM447 only or 2 week challenge treatments, so data for all spore concentrations was combined. Seedlings inoculated with PM447 (at any concentration) had significantly lower disease ratings than those inoculated with Westcott only or challenge inoculated. Westcott only and 2 week challenge treatments were not significantly different.

Figure 3. Two treated trees at the Jackson site. The tree on the left was inoculated with 10^2 spores of PM447 and the tree on the left with 10^1 spores. Both trees appear healthy with no symptoms of wilt. Photo was taken June 2006.

SECTION II

REGIONAL IMPACTS OF OAK WILT

OAK WILT IN THE APPALACHIANS

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ABSTRACT

Oak wilt was discovered a few decades after blight nearly eradicated the American chestnut, heightening concern that the oak resource in North America might be threatened similarly. Fortunately, in most Appalachian areas, the disease has spread slowly and erratically among a susceptible population of red oak species. This has occurred in spite of the existence of disease components that are common to areas of the United States where oak wilt is devastating; namely, a highly virulent causal pathogen, *Ceratocyctis fagacearum*, the existence of insects that have been identified as vectors, and the presence of root graft unions among susceptible oak species. A variety of hypotheses have been forwarded as to why the spread of oak wilt has been slow in the Appalachians. Certainly the diversity of hardwood species has limited tree-to-tree spread that is typical of areas in the upper Midwest and southcentral United States where *C. fagacearum* spreads freely through interconnected oak root systems. Likewise, many Appalachian sites possess rocky soils which have been speculated to restrict the development of functional root grafts, thereby further limiting tree-to-tree spread. Although known insect vectors are present in the Appalachians, their effectiveness is highly dependent on a variety of temporal conditions including the availability of inoculum as well as fresh wounds to serve as inoculation sites. All evidence suggests that the vectors are highly inefficient and proof of their relative importance in establishing new infections is circumstantial. In spite of the limited spread of oak wilt in the Appalachians, the disease can have very consequential influences in localized areas where it may smolder for decades, killing hundreds of oaks over time. The future of this disease could change rapidly if a more efficient vector were to emerge in this oak-rich region.

Key words: *Ceratocystis fagacearum,* disease management, insect vectors

Discovery of oak wilt (caused by *Ceratocystis fagacearum* (Bretz) Hunt) in Wisconsin in the 1940s and its subsequent diagnosis in the mid-Atlantic region resulted in immediate concern about the long-term effects of the disease on highly valued eastern oak resources. Extensive early surveys detected the disease from Pennsylvania southward to the Carolinas (True et al. 1960). Early assumptions were that this represented disease spread into new areas of the Appalachians. Obviously, the then relatively recent demise of the American chestnut from chestnut blight weighed heavily on the minds of conservationists and foresters of the day. This concern seemed

warranted as even today no forest pathogen is known to be as capable of killing members of the red oak group as efficiently as *Ceratocystis fagacearum*. However, with time, what initially was deemed spread into new forested areas appeared to reflect a more complete recognition of the disease rather than the expansion of the range of a recently emerging pathogen (MacDonald 1995).

Today, the incidence of oak wilt in much of the Appalachians can best be characterized as sporadic. In some locales, the disease is significant but in most regions it is rare or absent. For the Appalachians, the disease may have gone unnoted for decades had the symptomatology and causal agent not been described in the upper Midwest. Many trees die annually in any forest ecosystem and the relative few that contracted oak wilt in the Appalachians easily could have been discounted by anyone not familiar with the disease or not intentionally surveying for it.

Although the range of the disease frequently is depicted by county-by-county maps, this is not indicative of where the disease has its greatest impact. Data from West Virginia in the 1960s when annual surveys were conducted indicated that on average about 3,200 trees died statewide each year (Haynes 1995). However, the majority of infected trees were detected in the eastern panhandle of the state. Further, the disease has never been detected in several counties that are rich in susceptible oak populations. Likewise, oak wilt has never been detected to the northeast of the Susquehanna River in central Pennsylvania, yet susceptible oak populations and recognized vectors occur to the northeast of this area. One must ask why the disease has never spread into oak wilt-free areas in spite of the existence of all the necessary prerequisites for the disease, with the exception of the causal fungus.

Regions of the United States where *C. fagacearum* has been a successful pathogen possess significant populations of susceptible oak hosts, vectors capable of transmitting the pathogen, networks of inter-connected root systems among susceptible species, and appropriate environmental conditions to promote disease. Many of these factors differ vastly from region-to-region and the influence each factor exerts undoubtedly has resulted in the varied disease outcomes that are witnessed in different areas where oak wilt persists.

The two dominant components of oak wilt are the oak host and the fungus. Significant oak populations reside in the Appalachians with some areas comprised of more than 60% oaks (DiGiovanni 1990). Most prominent among the susceptible species are northern red oak (*Quercus rubra*), scarlet oak (*Q. coccinea*), and black oak (*Q. velutina*). Chestnut oak (*Q. montana*), a white oak considered intermediate in susceptibility, and several resistant white oak species, principally *Q. alba* (True et al. 1960) also are common. Although the forest ecosystems of the Appalachians have been altered considerably by a history of previous cuttings and fire, most hardwood species that existed 250 years ago remain today (Hicks 1997). Even though oak dominates many of the forests, it is joined by over 40 other species that make up the diverse Appalachian forests.

Even though molecular studies of *C. fagacearum* isolates from North America have demonstrated limited genetic variability, morphological and pathological variation have been observed among Appalachian isolates (Haynes 1976, Kurdyla et al. 1995). However, to the susceptible oak populations *C. fagacearum* infects, this variability may be irrelevant, as the fungus rapidly colonizes its host resulting almost certainly in sudden death.

TWO MAJOR FACTORS REGULATE DISEASE SPREAD

If both susceptible populations of oak species and the causal fungus exist in the Appalachians, what has limited its spread through the entire region creating a disease that is so sporadic in its

occurrence? Two major factors appear to be most responsible for the lack of significant dissemination of *C. fagacearum* in the Appalachians. The first is the frequency of root system spread. In areas of the upper Midwest and in Texas, root grafts among susceptible oaks provide a conduit for the movement of *C. fagacearum* from tree to tree (Appel 1995). Functional root grafts allow this vascular pathogen to move freely from infected host to an adjacent healthy tree. The frequency of root grafting in these high incidence areas appears to be tied to soil depth and texture, with higher rates of grafting occurring in lighter, sandier soils (MacDonald 1995). Likewise, the density and age similarities of like species that occur together clearly enhance the possibility of interconnected, functional grafts.

For the Appalachians, root grafting undoubtedly plays a role in maintaining centers of infection where like species are in close proximity to one another; however, the importance of the role of root grafts to pathogen transmission in the Appalachians often has been played down. The hypothesis remains that the rugged, often rocky soils typical of many areas in this region are detrimental to the development of functional root grafts thus restricting tree-to-tree spread (True et al. 1960). Likewise, the pathogen often can be isolated within a single tree or small group of trees if adjacent trees are not susceptible oaks or are other species. Thus, the inherent diversity of the species in the Appalachians likely minimizes root graft transmission as an avenue of pathogen spread. Further, there are instances where healthy oaks, within root graft distance of an infected tree, persist for 2-3 years before symptoms are detected (Rexrode 1978). No explanation exists for this delayed transmission phenomenon or whether root grafting even is involved when disease develops in an adjacent, previously healthy tree.

A second disease regulating factor relates to transmission by insect vectors. *C. fagacearum* largely is a xylem-limited organism. Its only phase outside its host is when it is acquired by a variety of insect vectors and is spread overland by those vectors. The strongest case for such overland transmission of the pathogen can be made for insects in the family Nitidulidae. These sap-feeding insects routinely have satisfied the prerequisites as vectors, particularly so when fresh wounds oozing sap occur during spring months (Merrill and French 1995). Beetles are lured to the fresh wounds on healthy oaks from the fragrant inoculum-producing mats on infected oaks, thereby spreading *C. fagacearum* to the healthy, but wounded trees.

Even though the role of the sap-feeding beetles has been demonstrated, their effectiveness has been questioned especially when evidence of mat production is rare or when spring wounding events have not occurred. Likewise, oak bark beetles of the genus *Pseudopityophthorus* are common in the Appalachians and their biology qualifies them as vectors, but evidence that they contribute significantly to overland spread is not convincing (Merrill and French 1995). There is little doubt that insects can and do vector *C. fagacearum* but apparently only low percentages of the many insects that have been studied actually acquire the fungus through their activities and, for those that do, their dispersal within a diverse hardwood forest makes them very inefficient disseminators. Clearly, for most vectors the critical sequence of events among the pathogen, host, and the environment that promotes vector efficiency seldom is met or the incidence of oak wilt in the Appalachians would be significantly greater.

OTHER DISEASE INFLUENCES

The topographic patterns of oak wilt spread have been studied in Pennsylvania and West Virginia particularly with respect to elevation and aspect and some with implications about insect vectors. For most studies, relationships of new infections to old have been difficult to establish as results often conflicted (MacDonald 1995). In some instances, new oak wilt

infections were found to occur more commonly on ridges and upper slopes, particularly on hillsides facing the prevailing wind currents. Presumably, fungal-laden insect vectors were carried downwind until they impacted stand openings or dominant trees protruding above the forest canopy, thereby transmitting the pathogen.

C. fagacearum has proven to be a virulent, aggressive, lethal, and systemic fungal pathogen. As a vascular pathogen, it largely is restricted to an existence within its host or by its relationship with vectors outside the infected host. Fortunately, the incidence of this disease appears to be rather static in the Appalachians and most regions of North America where oaks are a major forest component. In these regions either the pathogen has not been introduced successfully or conditions for its dissemination have not been fulfilled. Therefore, it would appear that for there to be a substantial change in disease incidence in most oak forest regions, a dramatic shift in vector relationships would be necessary. This could readily occur as pathways for the introduction of potential vectors from other continents abound and is made ever more likely by the increasing rates of international trade of logs and lumber. Obviously exotic vectors, capable of infesting sapwood of infected oaks, could establish new vector relationships and enhance pathogen spread in North America. Clearly for many oak regions, *C. fagacearum* is a pathogen in search of a better vector!

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OAK WILT IN THE NORTH CENTRAL REGION

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ABSTRACT

Oak wilt disease, caused by *Ceratocystis fagacearum*, is the single most important disease of oaks in the North Central region. Many factors, including pathogen distribution, forest stand composition, soil characteristics, and human activities, interact to result in variable levels of disease impact across the region. Opportunities for management are closely tied to activities that interrupt the spread of the oak wilt pathogen. Disruption of functional root grafts is most effectively accomplished by a vibratory plow. Overland spread is prevented by avoiding wounding and destroying potential spore-producing trees. Integration of these and other tools into a comprehensive management plan results in effective management of oak wilt. The USDA Forest Service is actively involved in oak wilt management in the region, through essential research, assistance to state programs, and management on federal lands.

Key words: *Ceratocystis fagacearum*, disease impacts, disease management

Oak wilt is the single most important disease of oaks in the North Central region. Although the origin of the causal agent *Ceratocystis fagacearum* (Bretz) Hunt is still unknown, most pathologists now believe that it is not native to the North Central region (Juzwik et al. 2008, Harrington, this proceedings). In fact, oak wilt has recently emerged as a very serious regional threat to oak health, and has been expanding in distribution and impact. Due to many interacting factors, the impact of the disease varies greatly among different locations. In the following pages we will discuss how these many factors affect the occurrence and management of oak wilt in the region. We will also discuss the distribution of the oak resource and the pathogen, variation in disease incidence severity, how biological factors affect opportunity for effective management, and the role of federal agencies in disease management within the North Central region.

OCCURRENCE AND SEVERITY OF OAK WILT DISEASE

Oak wilt is widely distributed throughout much of the region, yet it has not been reported from every county within the states of the North Central region, and there is considerable area with a significant red oak resource that does not have oak wilt disease (Fig. 1). Within the past 10 years,

oak wilt expansion (range extensions or intensification of disease within the known range) has specifically been noted in northern Wisconsin, the Upper and Lower Peninsulas of Michigan, southern Indiana, and urban developments north of the metropolitan area of Minneapolis/St. Paul, MN.

Oak wilt does not cause a consistently high level of impact across the region. Several biological factors that affect the spread and intensification of the disease through root graft connections and vector/host relationships account for this variation. These biological factors are intertwined with additional social factors, such as rural versus urban forests, changes in land use and forest cover, and long distance human movement of the pathogen. These factors are more fully addressed in a separate paper (Juzwik, this proceedings).

New infection centers of oak wilt usually arise when spore-contaminated nitidulids (sap beetles) visit fresh wounds on oaks (Gibbs and French 1980). If a tree becomes successfully infected, the fungus can spread to adjacent trees through grafted roots. Species composition and soil characteristics that affect frequency of root grafts determine the likelihood of this type of spread. In addition, ascospores and conidia from fungal mats can spread locally or longer distance via nitidulids. This type of spread leads to intensification of the disease on a site, or initiation of new sites.

In Missouri, older literature indicates that oak bark beetles may also be important in overland movement of the pathogen, and that competing microorganisms and weather factors may reduce the importance of fungal mats in spread of the disease (Rexrode and Jones 1970). However, three sap beetle species have recently been shown to be important vectors in the state (Hayslett, Juzwik and Moltzan 2008, Hayslett et al, this proceedings)). Studies in Minnesota have shown that, although fungal mats can be formed on oaks during the fall, the spring-produced mats are highly synchronized with nitidulid biology and behavior (Juzwik, Skalbeck and Neuman 2004). Furthermore, due to springwood vessel structure, the oak hosts are highly susceptible to successful infection by *C. fagacearum* during the spring. The synchronization of mat formation with nitidulid biology does not occur in fall (Juzwik et al. 2006).

Humans promote initiation of oak wilt centers by creating fresh wounds on oaks during highly susceptible periods. New infection centers in urban areas are well documented to be associated with construction and pruning wounds to oaks in the spring of the year. In forests, harvesting, thinning, road construction, or any other activity that wounds trees during the spring and early summer can lead to new oak wilt infections. This has recently been a problem on National Forest sites in Wisconsin and Michigan, where selective harvest and pruning activities in oak stands were implemented during the spring of the year, resulting in astonishing levels of new oak wilt disease (Joseph O'Brien, unpublished data, USDA Forest Service 2007).

The importance of the various biological factors (frequency of root grafts, stand composition, wounding, etc.) in spread and intensification of oak wilt is also affected by the actual distribution of the oak wilt pathogen. In areas where *C. fagacearum* is not known to occur, humans effect long-distance initiation of oak wilt centers through movement of infected material. Of particular concern is infected firewood or sawlogs that may harbor fungal mats. The presence of oak wilt disease in the Upper Peninsula of Michigan has been attributed to movement of infected firewood to seasonal-use properties (Bob Heyd, personal communication, Michigan DNR 2008).

OAK WILT MANAGEMENT ACROSS THE REGION

The primary tools for managing oak wilt are aimed at disrupting mechanisms of spread of the pathogen. Tools and management practices are available to prevent overland initiation of

infection centers via insect transmission of the pathogen, and to prevent below-ground intensification of infection centers via movement of the pathogen through root grafts. In addition, systemic fungicides are being used under certain situations to protect high-value trees. This section expounds on how each of these tools is used in the North Central region.

Prevention of Overland Spread

Avoidance of wounding during the highly susceptible spring period can prevent much overland insect transmission of *C. fagacearum* to healthy oaks. Wounds to oaks during this period require immediate treatment to prevent the cut surface from being infected by spores carried by nitidulid beetles. Even exposed stumps should be treated, as they serve as an open infection court.

In urban and suburban areas, prevention of human-made wounds on oaks is important. In the Midwest, there have been several effective public education campaigns to encourage people, e.g. "Don't prune in April, May and June." Many commercial arborists choose to avoid pruning during this time period. The susceptible period does vary across the region, and between years, so local knowledge is useful. It is also important to protect oaks from wounding at construction sites; this can often be accomplished by putting a fence or barrier around the oaks. This action can be effectively encouraged through education or community ordinances.

In woodlands, it is important to avoid forest disturbance activities during the susceptible period, and again, this can often be accomplished through education, local ordinances, or state guidelines. The Wisconsin DNR recently developed guidelines for harvesting in oak timberlands to minimize the potential for pathogen introduction to oak wilt-free stands or intensification in already affected stands (Wisconsin DNR 2007).

Another means to reduce overland spread is to reduce inoculum from fungal mats and pads. Trees from the red oak subgroup that have died from oak wilt in mid- to late-summer and have suitable sapwood moisture content for production of fungal mats during the following spring are called "potential spore producing trees," or PSPTs. White oaks are not considered to contribute significantly to risk of mat production. To effectively manage oak wilt, all PSPTs should be felled and portions greater than 2" diameter removed, treated, or destroyed prior to vector activity in the spring.

In most situations, removing the tree immediately after it dies is not recommended, because the pathogen may be pulled into the roots of healthy trees by transpiration of the adjacent living trees. The PSPT can be safely removed and treated to eliminate the possibility of spore mat production after vibratory plowing is completed on a site (usually in the fall). The stems can be safely utilized for timber products if they are removed from the site and processed prior to spring. They also can be utilized for firewood if they are debarked or are sealed with a tarp (to prevent nitidulid beetles from reaching the wood if spore mats are produced) from late winter until late summer of the same year. Acceptable methods to destroy wood from PSPTs include chipping, burning, or burying.

Prevention of Spread Through Grafted Roots

There are several tools to prevent transmission of *C. fagacearum* through root grafts. It has been demonstrated that in order to consistently halt the disease, functional grafts must be broken to a depth of 60 in. (1.5 m) (Bruhn and Heyd 1992). A vibratory plow equipped with a specialized blade is one of the most effective tools available for disrupting root connections between trees in the North Central region.

The placement of vibratory plow lines is critical to treatment success. Two methods are used in the North Central region. The "rule of thumb" method developed by D. W. French places a "primary" line outside the closest apparently healthy trees, so that there is a buffer zone of healthy trees between the oak wilt center and the plow line (French and Juzwik 1999). A secondary line can optionally be placed between the dead or dying trees and some or all of the buffer zone trees to try to 'save' some of these trees. A mathematical model developed by Bruhn, Pickens and Stanfield (1991) defines line placement with an equation that predicts the probability of the pathogen moving to an adjacent healthy tree within one year based on diameter of the healthy tree, diameter of the diseased tree, distance between the two trees, and soil type (Bruhn and Heyd 1992, Carlson and Martin 1996). With either method, oaks within the vibratory plow line are generally removed, either in a single preemptive action or over time as they succumb to oak wilt. Use of the mathematical equation method usually results in the removal of more trees than the "rule of thumb" method. Both methods are used within the North Central region, depending on the situation, location and preferences of the person defining line placement.

If a plow is not available, a trenching machine may be used to sever common roots, but the depth (usually 48") is inadequate to achieve consistent control. A backhoe can be used to dig an effective trench; however this is quite disruptive to a site.

Recently, on National Forest land, a previously untested method was used to control oak wilt center expansion in an area where uneven topography and rocky conditions precluded effective treatment with a vibratory plow. The method involves cutting and removing infected and adjacent trees and then using an excavator to rip out and overturn the stumps and root masses, a procedure coined "root rupture." In doing this, many of the root grafts are broken and diseased tissues are isolated from neighboring healthy oaks. Internal Forest Service documentation indicates that this method was greater than 90% effective in halting the spread of oak wilt (John Lampereur, personal communication, Chequamegon-Nicolet National Forest 2007).

The use of buffer zones alone, or simply cutting out oak wilt pockets, generally fails to halt the spread of oak wilt disease in the North Central region. Use of herbicides to kill a buffer zone of trees, with the hope that the root systems of killed trees will die and deteriorate quickly, has repeatedly been proposed as an alternative means of interfering with the graft connections of oak. Although some herbicide combinations have been identified that consistently kill oaks without sprouting, the root systems of the treated trees do not die quickly, making this method ineffective or impractical (Bruhn et al., 2003, Ed Hayes and Linda Haugen, MN DNR and USFS, respectively, personal observation, 2004).

Fungicide Injection

Systemic chemical treatment of high value oaks with propiconazole (PPZL) is a common practice in urban forests of the region. Preventive treatment of white and bur oaks within root grafting distance of infected oaks of the same species is effective in preventing wilt symptom development in treated trees (Eggers et al. 2005). Therapeutic treatment of *C. fagacearum*-infected white and bur oaks with $\leq$ 30% crown wilt symptoms has also been shown to prevent further wilting in such trees. Due to the observed success, commercial arborists generally only treat these species once they are infected.

Preventive treatment of red oak species within root grafting distance of pathogen-infected red oaks is common practice. In an evaluation of operational treatment by commercial arborists in the Minneapolis/St. Paul, MN, area, 39% of preventively treated red oaks died from oak wilt

over 5 years, but the deaths largely occurred 3 to 5 years after the single treatment (Eggers et al. 2005). Thus, many arborists commonly re-treat red oaks with PPZL two seasons after initial treatment.

In an experimental field trial involving paired treated and non-treated plots, differences in PPZL efficacy occurred by soil type/topography and by one versus two time treatments when compared to the control trees (Juzwik unpublished data). Results of a recent experimental study in red oaks suggests that PPZL likely suppresses disease development rather than eradicating *C. fagacearum* from roots or preventing root graft transmission (Blaedow and Juzwik 2008). Treatment of currently wilting red oaks is not advised as success in arresting wilt symptom development only occurred in trees exhibiting ≤ 25% crown wilt at the time of injection (Ward, Juzwik and Bernick 2005).

Management Conclusions

Just as the severity of the oak wilt pathogen varies across the region, the usefulness of these tools also varies. In areas with high incidence of root graft spread and deep sandy soils, use of the vibratory plow is highly successful, especially in parts of the region where vibratory plows and the necessary 60" blades are readily available. In other parts of the region, plows are harder to find, and 60" long blades are often not available.

Effective oak wilt control requires integration of tools to address all of the constraints and opportunities. The USDA Forest Service has produced three "How to" guides that provide valuable information to manage oak wilt (O'Brien et al. 1999, Pokorny 1999, Cervenka et al. 2001). The Forest Service has recently prepared other information products to assist with overall management of oak wilt disease.

In 2004, Forest Health Protection and Northern Research Station collaborated to produce a CD product to help communities implement an effective oak control program (Juzwik et al. 2004). This CD, entitled "Oak wilt: People and Trees, A Community Approach to Management", included powerpoint presentations, pdf files of relevant publications, and additional materials. The CD can be obtained from the Northern Research Station publications website, or from the Forest Service authors (Jennifer Juzwik and Linda Haugen).

In 2007, the Forest Service Forest Health Protection staff prepared "Northeastern Area Participation Guidelines for Oak Wilt Cooperative Prevention and Suppression Projects". This document provides guidance to help States and federal agencies implement effective oak wilt suppression projects. Suitable for a 3-ring binder, it includes appendices with detailed information on oak wilt biology, technical details of control measures, factors to consider when prioritizing treatment areas, and description of necessary documentation for federal projects. The guidelines are available from the US Forest Service Northeastern Area, State and Private Forestry, Forest Health Protection, St. Paul, MN.

THE ROLE OF FEDERAL AGENCIES IN OAK WILT CONTROL

The USDA Forest Service (FS) plays a vital role in the management and control of oak wilt disease in the North Central region. Its role is multifaceted and involves programs administered through State and Private Forestry and Research and Development.

State and Private Forestry programs, as authorized by Federal law, provide financial and technical assistance to protect state, private, and federal lands from the impacts of forest insects and diseases that pose a serious threat to the health and sustainability of urban and rural forest resources. Oak wilt is recognized as the single most important disease of oaks in the North

Central region, and the need to implement effective oak wilt management strategies has been identified as a high priority in several states.

To facilitate the effective management of oak wilt, State forestry agencies can use core-level funding provided by the FS annually, and they can apply for Cooperative Prevention and Suppression Grants. State agencies normally request Cooperative Prevention and Suppression funding because the anticipated costs of an eradication or suppression project exceed available state, local, or private funds. Federal land managers can also apply for Cooperative Prevention and Suppression Grants. To date, Oak Wilt Cooperative Prevention and Suppression Grants have been provided to State forestry agencies in MN, MI and WI; the Chequamegon-Nicolet and Huron-Manistee National Forests; and other federal agencies including the Department of Defense, Department of Interior, and Bureau of Indian Affairs.

State and Private Forestry staff also provides technical assistance to State and Federal partners. Technical assistance usually comes in the form of helping partners complete project documentation needed to meet NEPA requirements, including completion of biological evaluations, site specific environmental assessments, and consultations with the US Fish and Wildlife Serve (US F&WS) and the State Historic Preservation Office (SHPO).

Research and Development staff has dedicated significant efforts to conduct studies that have led to new or improved oak wilt management tools. A protocol was developed for monitoring flight activity of the predominant sap beetle vectors, particularly in early spring in Minnesota and Wisconsin (Kyhl et al. 2002). Sap beetle dispersal studies and frequencies of *C. fagacearum* contaminated beetle occurrence have been used to refine high, low and no risk time periods for tree pruning and harvesting activities (Ambourn, Juzwik and Moon 2005, Juzwik et al. 2006, Hayslett, Juzwik and Moltzan 2008). Experimental and observational studies on efficacy of PPZL for oak wilt control have resulted in improved guidelines (Eggers et al. 2005).

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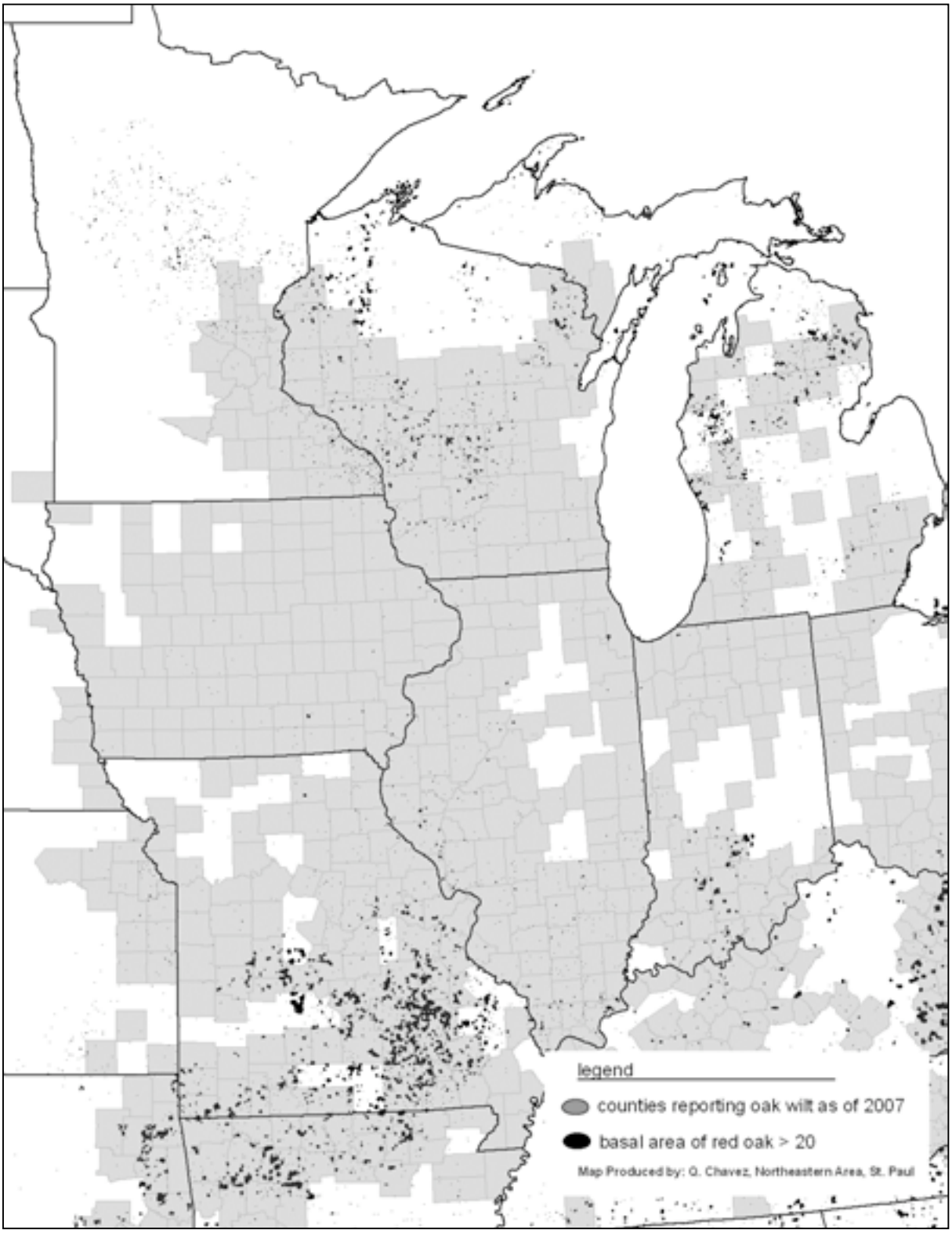

Figure 1. Distribution of oak wilt by county in 2007 and areas with basal area of red oak > 20 ft^2 according to Forest Inventory and Analysis (FIA) in the North Central region.

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OAK WILT: ITS IMPACT ON A GROWING TEXAS

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ABSTRACT

Since its official laboratory confirmation in the state of Texas in the early 1960s, the fungus which is responsible for the disease known as oak wilt (*Ceratocystis fagacearum*) has been confirmed in over 60 counties in central and west Texas. Since that time, explosive human population growth throughout central Texas has led to the fragmentation of traditionally large agricultural property holdings into smaller 10-50 acre "ranchettes." This fragmentation has been partly responsible for a transition in land use that moves away from traditional agriculture and toward a more multi-use management style. This new management regime also recognizes the added value that trees and tree canopy can provide. Not only do the introduction and preservation of trees satisfy these new multi-land use objectives which include recreation, aesthetics, and wildlife habitat, but trees directly contribute to an increase in overall property value. This newfound fondness of trees and their value to a growing population of tree-loving Texans also creates certain opportunities. The key lies in the ability of state forestry officials and their public and private partners to effectively increase the level of oak wilt awareness among these environmentally-conscious landowners. Currently, this is being addressed by creating one-stop sources of technical oak wilt information and assistance, and making them more easily accessible to the public by way of web-based services and GIS technology. By increasing the current levels of oak wilt awareness and providing the public with accurate and timely information on management of the disease, citizens and communities alike can be empowered to partner with state officials in better managing this statewide epidemic.

Key words: *Ceratocystis fagacearum*, disease management

Since the official laboratory confirmation of oak wilt in Texas in the early 1960s (Dooling 1961), and reports of oak disease centers long before that in many other areas of the state (Tabubenhaus 1934), forested areas throughout regions of central Texas (Fig. 1) have been severely impacted by this tree disease.

Oak wilt is caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt. The most obvious impact of this pathogen has been the loss of millions of Texas oaks over the last four decades (Lewis 1977, Appel and Maggio 1984). The continuing loss of these valuable tree resources has played a slow but steady role in altering many forest stewardship and land management decisions, if not permanently changing the perspectives that both rural and urban Texas communities have regarding the value and benefits of trees.

The occurrence and subsequent spread of the disease can be partly attributed to various human activities that result in the improper maintenance and wounding of trees (Craighead and Nelson 1960, French and Stienstra 1975, Juzwik and French 1983). Thus, oak wilt occurrences and impact have shown to be most significant in areas of greater human population.

TEXAS POPULATION GROWTH

Texas is growing, and growing fast (Fig. 2). To get a clear perspective on just how fast, consider the following statistics: During a period from 1990 to 2000, Texas' population grew by an estimated 3.9 million people, surpassing New York as the nation's second most populated state (Gilmer 2005). Currently, Texas ranks 8th in the US for percent population gain (22.8%). This considerable gain in net population now gives the state 13 of the top 100 fastest growing counties in the U.S. (Wilkins et al. 2003)

FRAGMENTATION

The steady increase in population directly results in an increased demand for land and housing. Fragmentation is a term commonly used by economists to describe the process in which traditionally large agricultural property holdings are broken down, or fragmented, into smaller 10-50 acre "ranchettes" (Fig. 3). To illustrate the impact of this trend; in a period from 1982 to 1997, Texas led all other states in the conversion of rural farming and ranching lands into some form of residential-based development. It is also estimated that the conversion of formerly agricultural-based landholdings into urban uses during this period exceeded 2.6 million acres. This is approximately double the rate of conversion compared to the previous 10 years (American Farmland Trust[1]).

A NEW BREED OF TEXAS LANDOWNER

The fragmentation of traditional agricultural land produces many smaller parcels. These parcels simply do not have the acreage needed to justify any type of potentially profitable farming, ranching or forestry practices. Most of these new landowners have little or very limited interest in working the land for a living – but are interested in land management from a non-conventional perspective. This new perspective has evolved into a new generation of land and forest stewardship for Texas landowners. This new type of stewardship has placed new emphasis on developing land for uses other than traditional agriculture.

Research has shown that the new landowners actively seek out properties that are away from the crowded urban areas; a place where they can escape the crowds and noise of urban life. These new land stewards commonly spend large sums of money in developing their properties to enhance various natural features such as wildlife habitat, picturesque view-scapes, and hiking trails. The only livestock that are managed on these properties are typically very small populations consistent with hobby farming or which allow the landowner to qualify for various agricultural tax exemptions or credits. In all - it's just enough to reconnect them to the spirit of the old West. In addition, a recent survey found that 80% of these new Texans stated that finding land for non-agricultural uses, like hunting, fishing and other recreation was a "very important" motive for their purchase (American Farmland Trust[2]).

As stated earlier, this new breed of Texas landowner seems to find great excitement in staking claim to a small piece of the rustic and colorful legacy of the state. These new landowners are younger, more educated and notably more affluent than their predecessors. According to a publication sponsored by the Federal Reserve Bank of Dallas (Gilmer 2005), this trend toward increased wealth can be further verified by monitoring levels of Texas employment and income growth records. Their data confirms that the Texas economy has outperformed the U.S. economy since 1969 (Fig. 4). And, by 2001, the state as a whole had raised its per capita income to 94 percent of the national average, up from 88 percent in 1969. Over the same period,

the average annual growth rate of per capita income was 2.3 percent for Texas versus 2.1 percent for the United States (Fig. 5) (Bryson 2006).

When it comes to the purchase of land in Texas, wealth does have its advantages. According to the American Farmland Trust[1], since 1994, residential development consisting of lots 10 acres in size or greater, has accounted for 55 percent of the land developed. Consequently, land prices are no longer driven by productivity of the land in terms of cattle and crops, but rather by its scenic and recreational value. These are natural features that have become the dominate factor in determining land value, and in many counties across Texas, these attributes have pushed prices to unprecedented levels (Gilliand 2007). The Texas Hill Country serves as a prime example; over the last decade, in a relatively isolated location of central Texas known as the Llano uplift (a region lacking a metropolitan area), the average market value for rural land increased by more than 86% over the last decade. This equates to approximately $514 per acre for land with an average agricultural value of $62 per acre (Wilkins et al. 2003).

VALUE OF TREES IN URBAN AND RURAL AREAS

The new style of stewardship also recognizes the importance of trees, both for their beauty and for the economic value they add to the property. Recognized methods of tree valuation have revealed that the presence of trees on a particular property may range from 13-19 percent of the total land value (Martin 1986). Unlike the economic contributions of trees, the aesthetic, social, communal, and environmental values are much more difficult to quantify, therefore, tend to be very subjective.

Although trees provide numerous aesthetic and economic benefits, they do come at a cost. For example; Texas landowners who are interested in reforesting an area denuded by oak wilt will quickly become aware that a sizable investment is required in order to purchase, plant, protect, and maintain the trees they desire. The largest expenditure besides the purchase and planting of new trees is the removal of the dead trees. This cost alone can be more than enough incentive for the landowner to increase his/her level of knowledge and awareness of not only oak wilt, but also of other potential forest and tree health issues that must be actively managed in order to protect the investment.

TREES FOR A GROWING TEXAS; RESPONSE OF STATE AND COMMUNITY FORESTRY PROGRAMS

As the population in Texas grows and land becomes more fragmented, the need for comprehensive tree and forest awareness programs increases. In 1982, in an attempt to address this need, the Texas Forest Service (TFS) initiated ane oak wilt demonstration project within selected counties of central Texas with funding from the United States Forest Service' Forest Health Protection Program. After 5 years (in 1988), the project was further expanded to include approximately 40 counties and became a federal suppression project. The Texas Forest Service strategically placed field offices throughout the region of central Texas to provide on-site technical and financial assistance to landowners battling this difficult tree disease. This was the first presence of TFS in this portion of the state (Billings et al. 2001).

To date, the Texas Oak Wilt Suppression Project has worked cooperatively with private and public partners in the field to effectively manage over 2,400 oak wilt centers (see Billings, these proceedings). TFS has placed further emphasis on increasing levels of public awareness regarding oak wilt and worked with multiple public and private partners to provide technical training for county extension volunteers and professional arborists. In 2005, TFS in cooperation

with the Lady Bird Johnson Wildflower Center in Austin created an Internet web page (http://www.texasoakwilt.org). Devoted exclusively to the identification and management of oak wilt in Texas, this web page received some 385,000 visitors in 2006.

Currently, seven multi-disciplined TFS foresters provide technical on-site services to landowners in six central Texas program delivery regions (Fig. 4). To complement the oak wilt management services, these foresters also have resources to provide technical assistance in areas of forest stewardship, reforestation, forest health, urban/community forestry, and other forestry-based practices. In 2007, the Texas Oak Wilt Suppression Project will have completed 20 years of service to the citizens of central Texas, one of the longest federal suppression projects on record.

In an on-going effort to get the word out to landowners throughout central Texas, specialists with TFS, Texas Cooperative Extension (now Texas AgriLife Extension Service), and Texas Agricultural Experiment Station (now Texas AgriLife Research) have trained various groups of Master Gardeners/Master Naturalists and ISA-certified arborists on the basics of oak wilt identification and management. These volunteers and professionals are now intercepting many of the numerous inquiries about oak wilt, lessening the burden on the few TFS foresters that deliver the Suppression Project.

CONCLUSION

By increasing the current levels of oak wilt awareness and empowering the public with accurate and timely information on management of the disease, citizens and communities alike can become partners with state officials in effectively addressing oak wilt at the local level and collectively managing a serious tree disease at the state level.

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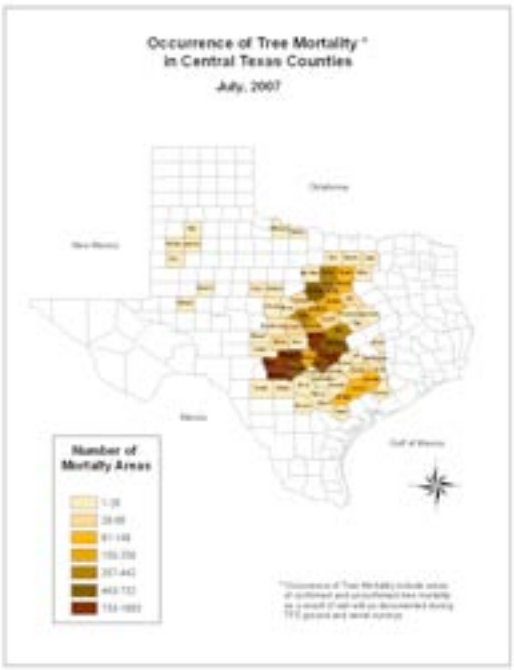


Figure 1: Tree mortality in Texas counties, July, 2007 (Texas Forest Service unpublished data).

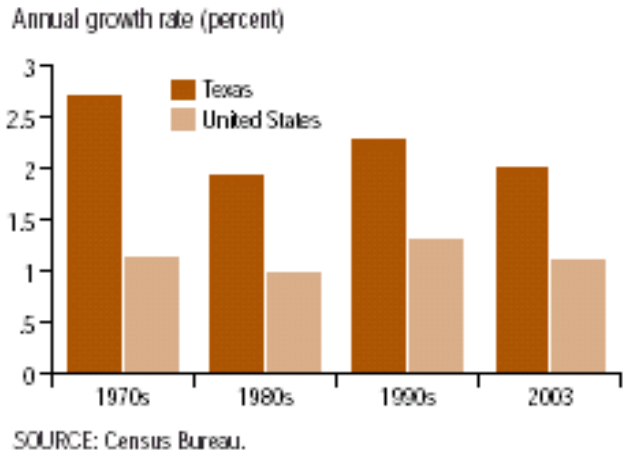
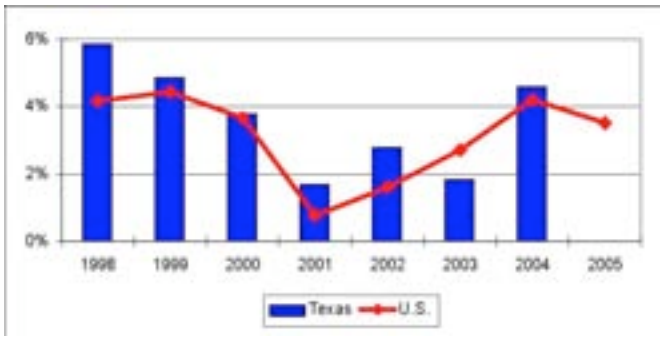


Figure 2: Texas and U.S. population growth, 1970-2003 (Source: U.S. Census Bureau).

Figure 3: Tree-friendly “ranchettes” located in central Texas (Photo by J.B. Rooni).



Sources: Economy.com and Wachovia Corp.

Figure 4: Texas and U.S. economic growth 1998 – 2005: Strong population growth has helped fuel the Texas economy.

	2001 per capita income (dollars)	Percent of U.S. level	Annual growth rate 1969–2001 (percent per year)
United States	30,413	100	2.1
Texas	28,472	94	2.3
Dallas–Fort Worth	33,247	109	2.2
Houston	34,916	115	2.5
Austin	31,611	104	2.8
San Antonio	26,887	88	2.3
Texas Triangle	32,897	108	2.4
Rest of Texas	21,357	70	1.8

SOURCES: Bureau of Economic Analysis; author's calculations.

Figure 5: Performance of regions of the Texas economy.

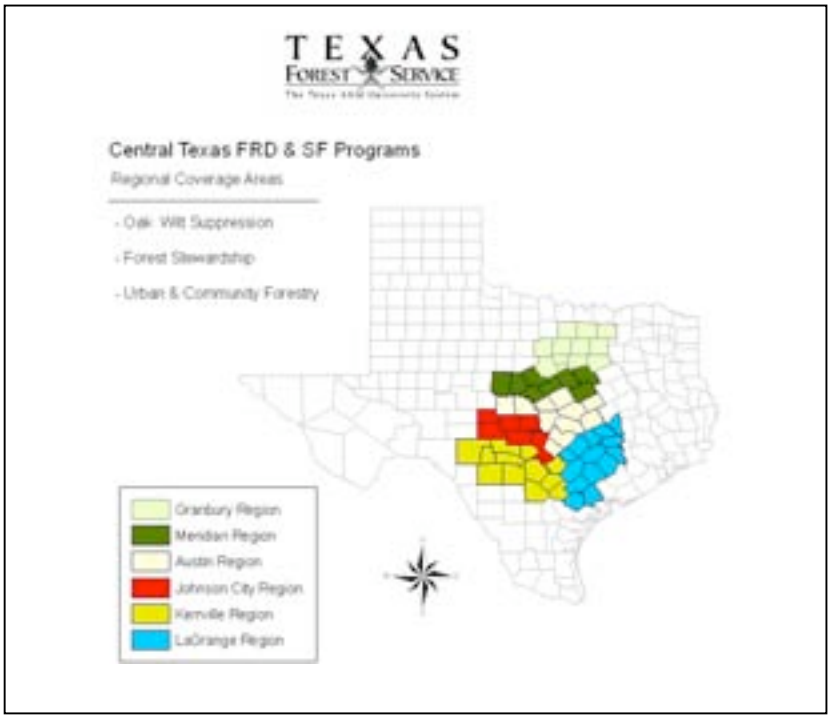


Figure 6: Texas Forest Service program delivery regions.

SECTION III

CURRENT AND POTENTIAL MANAGEMENT PRACTICES

INJECTION, INFUSION, AND SYSTEMIC MOVEMENT IN TREES

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ABSTRACT

Management of oak wilt often includes the use of systemic fungicides delivered by tree injection. Classic theory of sap movement being limited only to upward movement doesn't explain the efficacy of trunk-injected fungicides, whose site of action is in the root system. Movement of the xylem-mobile dyes, acid fuchsin, and saffranin O, after lower trunk/root flare injection, was found to occur both upward into the xylem of stems, twigs, and leaves, and downward into the xylem of woody roots, at most times of year. Similar patterns of movement of xylem-mobile dyes were observed on the following species tested: American chestnut, black birch, eastern hemlock, eastern white pine, red maple, red oak, weeping willow, white ash, and white birch. Downward movement of dye into root systems involved all ages of xylem tissues present within a root while upward movement was confined to the most recently formed xylem growth ring.

Key words: *Ceratocystis fagacearum*, dyes, fungicides, oak wilt

Trunk injection of systemic fungicides is often part of the management plan for the control of oak wilt (Appel 2007). Triazole fungicides delivered by trunk injection have been found to be effective in suppression of the oak wilt pathogen, *Ceratocystis fagacearum* (Wilson and Forse 1997, Wilson 2005). The ability of all systemic chemicals to suppress pathogens or control insect pests is dependent upon their systemic movement within the tree after injection. For systemic chemicals to move in the tree's vascular system is therefore dependent on the movement of sap within the tree.

The movement of sap from the roots to the top of tall trees has fascinated both scientists and others who wondered how a tree works. It is hypothesized that it is the loss of water (evaporation) from the leaves that causes a tension, or "pull", on many tiny water columns within wood (Campbell, Reece and Mitchell 1999). Since water is also cohesive, these combined forces can pull the water in a tree upward sometimes over 300 feet (100 meters) from the roots (Zimmerman 1983). This explanation for upward sap movement is known as the "cohesion-tension theory" and is widely accepted by tree scientists (Salisbury and Ross 1992). Since no corresponding theory has been proposed to explain the possibility of downward sap movement, it has often been concluded that sap flow only occurred in the upward direction.

However, experimental field data from those who studied sap movement in plants and vascular diseases of trees have reported evidence of downward movement for over 250 years (Banfield 1941). Many of these researchers used dyes or spore suspensions to track the downward movement of sap in trees. Banfield's studies on American elms demonstrated that both upward and downward movement occurred from injection points on the elm trees at equal speed. Extensive studies of sap movement by Greenidge (1958) using a sap mobile dye on a wide variety of trees, including American elm, balsam poplar, balsam fir, American beech, yellow birch, ironwood, sugar maple, white spruce, and white ash, supported the evidence of downward sap movement found earlier by Banfield (1941) and others.

Further evidence of downward movement of injected chemicals came form microinjection studies with the antibiotic oxytetracycline, which has been used to relieve symptoms of numerous bacterial diseases of trees, including bacterial leaf scorch (Kostka, Tattar and Sherald 1985), peach X-disease (Cooley, Tattar and Schieffer 1992), and lethal yellows of coconut palm (*Cocos nucifera*) (McCoy 1983). High populations of systemic bacteria within the root system have been associated with diseases caused by systemic bacteria (Sinclair, Lyons and Johnson 1987, Cha and Tattar 1991, Blanchard and Tattar 1997). However, it has puzzled scientists how a systemic chemotherapeutant, such as oxytetracycline, or a systemic fungicide, such as propiconazole, could be effective if xylem movement of injected materials only occurred in the upward direction. Preliminary studies by Tattar and Tattar (1999) presented evidence for downward movement in the xylem of trees following trunk injection with the use of xylem-mobile dyes.

The objectives of this study were (1) to determine the direction and magnitude of movement of trunk-injected materials within the xylem of trees using xylem-mobile dyes and (2) to determine how time of year of injection influences dye movement.

MATERIALS AND METHODS

The trees used in these studies were growing in the Shade Tree Laboratory Nursery in Hadley, MA and in the Cadwell Memorial Forest in Pelham, MA. Both these research facilities are part of the University of Massachusetts at Amherst. The trees ranged in size from 2 inches (5 cm) to 10 inches (25 cm) in stem diameter at 4.5 feet (1.4 meters) above ground. The following species were injected: red maple, *Acer rubrum*, eastern white pine, *Pinus strobus*, red oak, *Quercus rubra*, eastern hemlock, *Tsuga canadensis*, white birch, *Betula alba*, black birch, *B. lenta*, American chestnut, *Castanea dentata*, white ash, *Franxinus americana*, and weeping willow, *Salix babylonica*.

Tree injection wounds were made with a battery-powered drill (800 rpm) using an 11/64 inch (6 mm) high speed steel drill bit. Injection holes were made in the lower trunk and root flare areas and hole depths were between 1/4 inch (6 mm) and 1/2 inch (12 mm). In one study conducted during the 1997 summer season, however, injection wounds were made at 4.5 feet (1.4 meters) above ground to American chestnut and red oak trees. An unpressurized glass reservoir container, with an exit port at the bottom of the container, was filled with 25 to 50 ml of dye solution. The reservoir delivery system was attached via plastic (Tygon) tubing to a hollow plastic tube which was inserted into the injection wound, immediately after a drill-hole injection was made. The following xylem-mobile dyes at 2% w/v were each used during these experiments: acid fuchsin, gentian violet, and safranin O.

Trees were injected with the test dye solutions in late spring during leaf expansion through mid fall after leaf drop. Experiments were conducted over an 8-year period from 1998 through 2005. Dye injection studies were started either from 0800 to 1000, or from 1400 to 1600. In most experiments, injectors were left in the tree for 24 hours. Trees were harvested immediately after injector removal. In some experiments, dyes were injected in the morning, the experiments were terminated approximately 6 hours after injection, and trees were harvested in the afternoon. While in other experiments, trees were injected in the afternoon and harvested the next morning, approximately 16 hours after injection. Soil temperature was measured at 5 cm (2 inches) below ground, using a soil thermometer, at the starting time of each injection.

On most trees 10 cm (4 inches) and smaller in diameter, the woody roots were severed with a root ax and/or hand saw and the entire tree was examined. Soil was removed from roots by

washing and the bark was peeled from the woody roots and stem. In some larger trees the root flare was exposed by removal of soil and only the large roots were cut with a chainsaw, approximately 20 to 50 cm (8 to 20 inches) from the trunk. All stem and root sections were photographed as soon as possible after the bark was removed.

Dye movement in both upward and downward directions in the xylem was assessed by visual examination of the leaves and by estimating the amount of xylem tissue stained by the injected dye after the bark was removed. We were usually able to follow patterns of dye movement throughout the test trees from the leaves to the roots.

RESULTS

The first studies were conducted in the fall during and after onset of leaf coloration and continued after leaf drop of deciduous trees. Dye patterns, regardless of species, were always bimodal, with some dye movement upward into the stem and downward into the roots from the injection sites at the root flare. Dye movement in the initial studies was approximately split between upward movement and downward movement. Later fall studies displayed progressively greater downward dye movement as soil temperatures declined from approximately 15°C (60°F) to 5°C (40°F). After complete natural leaf fall, dye movement was primarily downward until experiments were terminated in early November. These dye patterns were consistent with all the species studied, in both deciduous hardwoods and conifers. In addition, the dye patterns were also similar regardless of the dye solution used. Acid fuchsin and saffranin O were most easily observed.

Studies were also conducted during leaf expansion in late spring and continued into the summer when full leaf size of deciduous trees was attained. Our initial results were similar to early fall studies, with dye movement evenly split between upward and downward directions. Experiments conducted during summer were remarkably similar to those of late spring, but even with a progressive increase in upward movement, we always noted substantial downward movement. During moisture limiting soil conditions, downward movement was found to increase. Cross sections of roots revealed dye movement into several years of xylem tissue while stem cross sections of the same trees revealed dye confined only to springwood vessels of the current growth ring.

In an attempt to determine the speed of downward movement or upward movement, dye reservoirs were left on trees for fewer than 24 hours. However, even when dye reservoirs were in place for only 6 hours during day experiments and 16 hours during night experiments, bimodal movement was found. We noted on several occasions that, after downward movement into the roots, the injected dye would then reverse direction in the roots and progress upward on the opposite side of the stem.

In a study of the effect of the height of injection on systemic dye movement, American chestnut and red oak were injected at 4.5 feet (1.4 meters) above ground. Most of the acid fuchsin dye moved upward into the branches and foliage and only small amounts of dye moved downward, compared with similar trees that were injected at the root flare on the same dates and times.

DISCUSSION

Downward movement within xylem can be explained by the normal condition of the functioning xylem elements, which are under negative pressure or tension, and is consistent with the cohesion-tension theory of xylem movement. A break in the xylem elements, due to an injection

wound, would allow movement of the injected solution in either upward and/or downward directions according to the forces within the xylem elements at the time of injection.

The results of this study agree with those of Banfield (1941), Greenidge (1955), and others who reported downward movement of dyes and fungal spores in the xylem of many tree species. The findings of the current study, based on dye delivery by trunk injection, combined with those of earlier researchers, can help to explain how materials injected into the sap stream at the root flare can have efficacy in the root systems of trees. This information is especially useful in explaining the control of root problems achieved using trunk injection of antibiotics, fungicides, insecticides, and micronutrients during the growing season with active leaf transpiration. For example, these findings may help to explain why trunk injection was found to be effective in the treatment of pathogens that are primarily transmitted through the root system, such as oak wilt (caused by *Ceratocystis fagacearum*), since the early 1990s (Osterbauer and French 1992, Appel 1994).

Osterbauer and French (1992) reported that location of injection sites on the root flare may have resulted in movement of the propiconizole into the root system since they could not detect the fungicide above a height of 3.0 meters. Although these researchers did not conduct any propiconizole assays of root tissues, results obtained in this study would support their conjecture of downward movement of the injected fungicide. One may also conclude from the results of the current study that downward movement of injected systemic chemicals is favored by placing injection sites in the root-flare zone.

Multi-year xylem sap distribution in roots would appear to explain vascular disease control beyond one growing season achieved using injectable fungicides, such as that reported by Osterbauer and French (1992) with propiconizole. Dye movement was found across the entire cross section of root xylem following lower trunk injection . It appears that portions of trunk-injected materials are transported downward into the roots and are then transported upward in the sapstream in the following season or seasons. This theory could also account for the efficacy of fall-injected materials in the following spring.

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EFFECTIVE LONGEVITY OF PROPICONAZOLE FOLLOWING INJECTION INTO *QUERCUS RUBRA*

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ABSTRACT

Propiconazole was injected into *Quercus rubra* to determine duration of efficacy against *Ceratocystis fagacearum*, the fungus that induces oak wilt. Eleven and 13 trees were treated preventatively in 2002 and 2003 respectively; these trees were subsequently inoculated with a conidial suspension of *C. fagacearum* at 0, 9.5, 14, 21.5, 23, 24, or 34 months following fungicide injection. Five- to six-foot (1.5- to 1.8-m) deep trenches isolated treatment groups. Control trees were located throughout the site and included trees either injected with fungicide, inoculated, or untreated and non-inoculated. Propiconazole-injected trees inoculated in May 2005, as late as 34 months following initial fungicide treatments, did not express wilt symptoms for at least three months; however, all untreated, inoculated control trees developed symptoms within six weeks. As of August 2006, over one year after final inoculations, 14 of the 24 treated and inoculated trees (including five of eleven trees inoculated at 34 months) remained symptomless. Results suggest that inhibition of *C. fagacearum* may occur even at 34 months post-injection.

Key words: *Ceratocystis fagacearum*, fungicide, oak wilt

Oak wilt is a lethal disease of oaks caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt. The pathogen invades the xylem inducing tylosis and gummosis in the host, which, in addition to fungal material, results in the blockage of water through sap tissues. Overland spread of *C. fagacearum* occurs via insect vectors, while local spread is primarily through root grafts that form between neighboring trees of the same species. Intravascular injections with a wide variety of antibiotics and fungicides have had limited success in treating or preventing oak wilt (Phelps, Kuntz and Ross 1966, Appel 1995). In 1987, however, Appel determined that live oaks (*Quercus fusiformis* and *Q. virginiana*) injected with the triazole fungicide, propiconazole, had significantly lower disease levels compared to untreated trees. Based on this study, propiconazole injection was deemed to be an effective treatment for oak wilt and was registered for use on live oaks in Texas (Appel 1995).

Osterbauer et al. (1992 and 1994) subsequently studied injection treatments in other species of red oak (subgenus *Erythrobalanus*) and white oak (subgenus *Leucobalanus*) in Minnesota and found that propiconazole could protect a treated tree for up to two years against root-graft spread. Additional research on propiconazole injection treatments has shown that white oaks typically respond well to fungicide injection and can often be treated therapeutically, whereas red oaks often succumb to wilt despite treatment if they already are infected (Osterbauer and French 1992, Osterbauer, Salisbury and French 1994, Eggers et al. 2005). Therefore, red oak injections are usually limited to high value trees with little or no symptoms of disease. Due to the high cost of treating trees (an average tree may cost a few hundred dollars to treat including chemical and

labor costs), additional knowledge regarding the activity of propiconazole within a tree in relation to its distribution and to the distribution of the pathogen within the host is necessary for effective management strategies.

As the fungus can remain viable for several years in the roots of wilted trees, and spread through root grafts may take several years to occur, it is difficult to predict where the pathogen is within the root system at any given time (Yount 1955, Rexrode 1978). In addition, disease progression in root-infected trees is often delayed in comparison to trees inoculated above ground (Cobb, Fergus and Stambaugh 1965). Therefore, symptomless trees in naturally-infected stands may or may not already have the pathogen within their roots. Previous research has focused on the efficacy of propiconazole injection against root-graft transmission of *C. fagacearum* in stands where oak wilt was present. We investigated the longevity of propiconazole activity in a wilt-free oak stand in Michigan where root-graft transmission was prevented by trenching prior to experimentation.

MATERIALS AND METHODS

All oaks (*Quercus rubra*) used in the study were located on the same site on the Michigan State University campus and ranged from 16 to 51 cm diameter at breast height (dbh), with the average dbh equal to 30 cm (standard error ± 2.4). Trees at the site were arranged in six rows and had been established for several decades. The soil consisted primarily of Colwood-Brookston loam (62%) with Capac loam comprising the rest (38%). These series are characterized by deep, poorly drained, fine loamy soil. Five- to six-foot (1.5 to 1.8-m) deep trench lines were made using a Davis Fleetline 70+4 trencher, which isolated treatment groups by disrupting potential root grafts (Figs. 1 and 2). The experiment was replicated twice, first in July 2002 (Replicate 1) and then in June 2003 (Replicate 2). Forty-two trees were utilized in the study and an additional 21 trees at the site were maintained as negative controls (untreated and non-inoculated). Treatments consisted of trees injected with propiconazole and then inoculated with a wild-type *C. fagacearum* isolate, with the time interval between chemical injection and fungicide inoculation ranging from 0 (inoculated immediately following fungicide injection) to 34 months. Untreated, positive control trees were inoculated with the wild-type strain throughout the course of the study.

Since none of the fungicide-treated and inoculated trees from Replicate 1 developed symptoms by 2005, these 11 trees were inoculated a second time and included into a 34-month inoculation treatment. Therefore, at the conclusion of this study, all non-control trees from Replicate 1 had been inoculated twice: once at 0, 9.5, 14, 21.5, or 24 months after injection, and then again at 34 months post injection. In Replicate 2, trees from treatment groups 0, 9.5, and 14 months also remained symptomless in 2005 and so were incorporated into the 23-month treatment in 2005. Thus, the 23-month treatment group in Replicate 2 consisted of seven previously-inoculated trees and three trees that were only inoculated once, 23 months after fungicide treatment. Trees from Replicate 2, 24-month treatment, were inoculated only once at 24 months following injection.

Fungicide treatments with Alamo® (propiconazole 14.3%) were carried out via pressurized macro-injection into root flares with a 12V Flowjet pump according to the fungicide product label (Novartis Crop Protection, Greensboro, NC) in July 2002 (Replicate 1) and June 2003 (Replicate 2) (Fig. 3). Injection pressure was maintained at 20 pounds per square inch (psi). Trees were treated with 20 ml of fungicide diluted in one liter of water per 2.5 cm (1 inch) of tree dbh (2.8 g active ingredient per 2.5 cm dbh or 0.09 oz per inch dbh), which is the manufacturer's

recommended dosage for trees under high disease pressure. Injection wounds were painted with wound paint the day after injection before being covered again with soil.

In general, trees absorbed the fungicide solution within a few hours; however, a few trees took much longer and often did not take up the full amount of product even when pressure was increased or the injection apparatus was left connected to the tree overnight. These trees typically received less than two-thirds of the attempted injection amount. Though these trees were retained in the study, their lack of full absorption was noted and any significant variations in results were considered.

Inoculations were performed with one wild-type strain, "Westcott", recovered from a diseased tree in Ogemaw County, Michigan in 2001. Conidia from Westcott cultures grown on plates containing potato-dextrose agar (PDA) were collected by placing 1-2 ml of distilled water onto the plate and gently rubbing the surface with a glass rod. The resulting suspension was strained through Miracloth™ and the concentration was adjusted to 10^5 conidia/ml with water and 20% glycerol. This suspension was divided into 1 ml aliquots and maintained at -80º C. The conidial suspension was thawed at room temperature for one hour prior to inoculation studies. Viability of spores was periodically assessed by serial dilution onto Petri dishes containing PDA; spore viability was consistently greater than 90%. For tree inoculations, a 2.5 cm-deep hole was drilled into the north side of the trunk at 1.5 m above ground with a 6 mm (¼ inch) bit. One ml of the 10^5 conidia/ml suspension was then placed in the hole with a pipette. The suspension was generally absorbed within 5-10 minutes, and holes were subsequently covered with tape to prevent insects from entering the wound.

Trees were visually assessed monthly (May through October) for symptoms of oak wilt to determine the timing between inoculation and initial symptom development. Symptomatic branches from trees expressing disease symptoms were sampled for the presence of *C. fagacearum* by flame sterilizing samples after dipping in 90% ethanol, removing the outer bark, and then placing pieces of sap wood onto plates containing either PDA or glucose-phenylalanine agar. Final inspection of trees was done in early August 2006, 15 months after final inoculations. For the purposes of this study, trees were rated as either diseased (1) or healthy (0).

The relationship between treatment parameters and disease development was analyzed by exact conditional logistic regression using the LOGISTIC procedure in SAS version 9.1 software SAS Institute Inc., Cary, NC). Disease was modeled as a function of replicate (1 or 2), month (when inoculation occurred), the number of times a tree was inoculated (once or twice), and whether trees received fungicide prior to injection. Significance of treatment variables to the model was determined according to a Score test and exact parameter estimates were analyzed to determine the type of effect each predictor variable had on disease occurrence. A p value ≤ 0.05 was used to determine statistical significance.

RESULTS AND DISCUSSION

All 17 positive control trees from both replicates developed wilt symptoms within six weeks following inoculation and were completely wilted within the same year (Table 1). Thirteen of the 21 negative control trees were incorporated into other studies in 2005 leaving eight non-inoculated controls in 2006. None of these trees developed wilt symptoms over the course of the study, indicating that the trench lines initially established in 2002 remained effective and that insects were not moving inoculum.

Possible wilt symptoms on treated trees first appeared in late fall of 2005, but as these symptoms developed just before fall coloration, wilt was not confirmed until the following year.

All symptomatic trees sampled in 2006 were positive for *C. fagacearum*. Of the propiconazole-treated trees from Replicate 1, only six of eleven trees showed disease symptoms in 2006. All six symptomatic trees had been inoculated twice: two at 14 and 34 months after fungicide injection, two at 21.5 and 34 months, and two at 24 and 34 months.

Four of the 13 treated and inoculated trees from Replicate 2 displayed symptoms in 2006: one of the three trees that had been inoculated once only at 23 months and three of the seven trees that were inoculated twice (one at 0 and 23 months and two at 14 and 23 months) All four of the trees from Replicate 2 that expressed wilt symptoms in 2006 failed to absorb the full amount of fungicide when injection was attempted. Only one tree in this study that did not take up the full amount of propiconazole did not develop wilt symptoms. Two of the three trees inoculated once at 23 months and all of the 24 month trees (Replicate 2) remained symptomless over one year after inoculation. Yet, the untreated control trees inoculated at 24 months developed wilt symptoms in 2005, the same year they were inoculated.

All fungicide-treated trees that developed wilt had delayed symptom development both initially and after symptoms appeared. Symptoms were not obvious for at least 3-13 months after inoculation and were confined to scattered branches where the disease progressed slowly. This is contrary to what was observed in the untreated control trees, which expressed symptoms within six weeks following inoculation that progressed rapidly from the top of the crown, downward.

The number of times a tree was inoculated and whether or not a tree received fungicide prior to inoculation significantly contributed to the disease model; however, month ($p = 0.43$) and replicate ($p = 0.28$) were not significant explanatory variables and were excluded from the model. Based on parameter estimates, trees that did not receive a fungicide injection and those trees inoculated twice had greater incidence of disease, while fungicide-treated trees (regardless of month) had decreased disease incidence. The null hypothesis that one inoculation had no effect on disease cannot be rejected ($p = 1.00$).

These results indicate that propiconazole potentially remains effective for at least 24 months and provides some level of protection up to 34 months post-injection. Interestingly, Osterbauer and French (1992) were only able to detect propiconazole using a thin layer chromatography assay up to 12 months following injection. A double (instead of a single) band was observed in sample lanes at 16-18 months post-injection, similar to that found in the fungicide standard lanes when older supplies of propiconazole were analyzed, suggesting degradation of the product. At 20 months post-injection, no propiconazole was detected in any samples. Our results demonstrate that the product may still inhibit fungal growth even after 34 months and that the amount of propiconazole injected may influence the length of efficacy of the product, as Osterbauer and French (1992) used much lower rates in their studies. Additionally, the TLC assay they used may not have been sensitive to low levels of propiconazole that would still affect *C. fagacearum.*

Propiconazole is a triazole-fungicide, one of the classes of sterol-biosynthesis inhibiting fungicides. Wilson and Forse (1997) determined that propiconazole, at sufficiently high levels, was fungicidal to *C. fagacearum*. Therefore, in this study, the pathogen was potentially killed due to high initial levels of the fungicide in trees inoculated soon after injection. However, at lower concentrations of sterol biosynthesis inhibitors, the inhibition of fungal spore germination is incomplete (Kuck and Scheinpflug 1986, Latteur and Jansen 2002, Nogueira et al. 2002). Thus, propiconazole may delay active colonization of the fungus until it degrades to low enough

levels, at which point the pathogen can spread throughout the tree. Incomplete distribution of the fungicide within a tree would also contribute to this effect.

In addition to its fungistatic effects, triazoles, including propiconazole, are known to have plant growth regulating properties (Kuck and Scheinpflug 1986, Wetztein et al. 2002, Hanson et al. 2003). Phelps, Kuntz and Ross (1966) reported that indole 3-acetic acid, a natural growth regulator, delayed symptom development in northern pin oak (*Q. ellipsoidalis*) up to 12 months or more when injected into the trunk. Although indole 3-acetic acid is an auxin and thus stimulates growth, whereas propiconazole has a growth retardation effect, by changing the balance of growth regulation in the plant (perhaps enhancing the tree's ability to cope with stress or interfering with the production of tyloses in response to the pathogen), disease development is affected. Thus, it is possible that propiconazole works in two ways to inhibit disease development – first by interfering in ergosterol-biosynthesis and secondly by affecting growth regulation within the host.

Interestingly, the effects of propiconazole on disease development are similar to those found with other compounds tested for the control of wilt. Phelps, Kuntz and Ross's (1966) research on northern pin oak shows that a few antibiotics and/or chemicals prolonged the incubation period of the disease up to 24 months. The pattern of symptom development on such treated trees differed from untreated trees in that wilt symptoms developed slowly, often branch by branch, and sometimes over one to two years. Similar results of a temporary delay effect were found with trials using thiabendazole for wilt (Appel 1995). We also observed this effect on the treated trees in our plot that eventually developed symptoms: symptoms were initially confined to particular scattered branches and progressed much more slowly than would be expected (in comparison to inoculated controls). Phelps, Kuntz and Ross (1966) also reported that, despite a prolonged incubation period, the fungus was isolated from 75% of symptomless trees 12 months after inoculation. This demonstrates the ability of the fungus to remain within a tree without inciting disease, further supporting the idea that propiconazole may have ultimately only delayed disease development in our plot. However, given that approximately half the trees inoculated for a second time at 34 months post-injection did not develop symptoms over one year later, it may be that by suppressing pathogen growth long enough, a tree could fundamentally be protected.

As the majority of research on oak wilt has focused on the host-pathogen interaction above ground, there remain many unanswered questions regarding the movement and colonization of the pathogen in the root systems. Evidence of pathogen movement through root grafts may take one to three years (Rexrode 1978) and seemingly dormant disease centers may begin wilting again after several years, presumably due to root-graft spread. Additionally, root-inoculated trees often display delayed wilt symptoms up to one year from inoculation (Cobb, Fergus and Stambaugh 1965). Thus, it is difficult to determine when and how the pathogen enters the root system and what happens once it is there. Defensive reactions in response to *C. fagacearum* are less extensive in the roots than in other parts of a tree (Struckmeyer et al. 1953). This suggests that the pathogen may be able to colonize parts of the root system, which has implications for disease development in fungicide-treated trees. There is evidence that propiconazole is distributed to the root system of injected trees (Tattar and Tattar 1999, Blaedow et al. 2005), but it is unclear to what extent and how this affects pathogen growth and movement within the root system.

Natural infection with *C. fagacearum* is most likely to occur through branch wounds or root graft movement; thus, the results from this study must be interpreted accordingly. Our experiment tested the effectiveness of propiconazole injection against non-root graft spread of

the pathogen as trees were inoculated in the bole. The observed inhibitory effect may break down with natural overland infections, which probably occur in the crown, as it is believed that the fungicide is not translocated or distributed evenly throughout the upper canopy (Osterbauer and French 1992). While it has been documented that bole inoculations have greater inoculation success than crown-inoculated trees (Jones 1964, Cobb, Fergus and Stambaugh 1965), it is also probable that the greatest amount of fungicide was distributed within the trunk. Therefore, the observed delay in symptom development may or may not be related to the initial distribution of the fungicide in relation to the inoculation site, underscoring the need for further clarification of this relationship.

CONCLUSION

The possible dual inhibitory effects of propiconazole and advances in delivery via macro-injection have made propiconazole a promising fungicide treatment for oak wilt. However, since the early work by Osterbauer and French (1992) on propiconazole injections in red oaks, it has been apparent that red oak treatments are somewhat unpredictable. Our results show that propiconazole injection was an effective preventative treatment for oak wilt in some cases up to 34 months following injection; however, disease pressure apparently affects the duration of efficacy, as intimated by disease occurrence in trees receiving two inoculations. All but one of the treated trees that developed symptoms had been inoculated twice and did not express obvious symptoms for over a year after final inoculations, whereas all positive control trees developed symptoms within six weeks, indicating that treated trees inoculated at the same time were delayed in symptom development. Additionally, all symptomatic trees from Replicate 2 had not taken up the full amount of fungicide administered. Given these results, the effective longevity of propiconazole appears to be dependent on several factors including the amount injected, the level of disease pressure, where the pathogen enters a tree, and the relative distribution of the pathogen and fungicide within a host tree.

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Table 1. Proportion of wilting trees in August 2006 per replicate. Treatments consisted of trees injected with propiconazole and then inoculated with *Ceratocystis fagacearum* at A) 0 (inoculated same day as injected), 9.5, 14, 21.5, 23, or 24 months later and B) once at 0, 9.5, 14, 21.5, or 24 months and a second time at 23 or 34 months. Positive, untreated control trees were inoculated at the respective time (in months) after experiments began. Replicate 1 began in July 2002, while replicate 2 was initiated in June 2003. A '-' indicates treatment was not included in that replicate.

A

	Treatment (months)							
							Control trees	
	0	***9.5***	***14***	***21.5***	***23***	***24***	***0***	***24***
Rep 1	0/3	0/2	0/2	0/2	-	0/2	4/4	3/3
Rep 2	0/2	0/2	0/3	-	1/3	0/3	4/4	2/2

B

	Treatment (months)			
			Control trees	
	23	***34***	***24***	***34***
Rep 1	-	6/11	3/3	4/4
Rep 2	3/7	-	2/2	-

Figure 1. Trenches, dug with a Davis Fleetline 70+4 trencher, were used to isolate treatment groups.

Figure 2. Five to six-feet-deep (1.5-1.8 m) trenches break potential root grafts between neighboring trees. Trenches are approximately 4-6 in. (10-15 cm) wide.

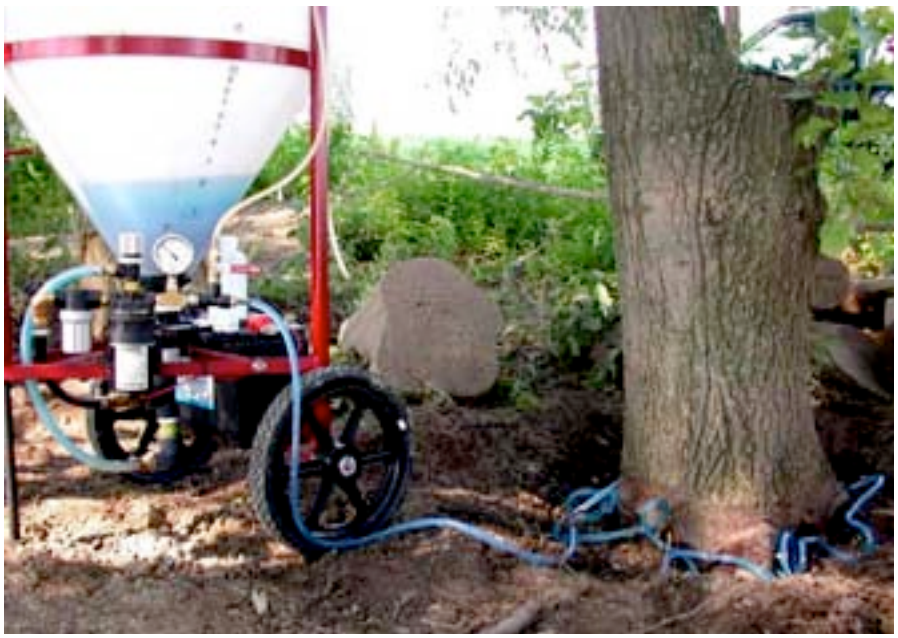

Figure 3. Injection apparatus used for propiconazole injection treatments. The fungicide solution was pumped out of the storage tank and into 2.5 cm-deep holes drilled into the xylem of the root flares.

ATTEMPTS TO DEVELOP AN OAK WILT RESISTANT LIVE OAK

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ABSTRACT

Oak wilt, caused by *Ceratocystis fagacearum,* was confirmed in Texas in 1961 and has since been found in 60 Texas counties. Much of the epidemiology of oak wilt in Texas has been elucidated. Protocols have been developed to hinder local and long distance spread of the pathogen, to treat infected high-value live oak trees, and to protect high-value trees situated next to diseased trees. What is lacking in the arsenal to defeat this epidemic in Texas is resistant live oak (*Quercus fusiformis*) stock. One unique aspect of the Texas epidemic is the apparent, partial resistance in live oak to the disease. This suggests that either genetic or environmental components are responsible for variable survivability to the pathogen. Previous research at Texas A&M University found evidence for heritable, genetically-determined resistance and for phenotypic markers (allozymes) associated with disease tolerance. In order to expand on these findings, we used clone and seedling crops to test for genetically- determined resistance to the pathogen. In one study, resistance of clone groups and seedling groups was tested for a potential correlation with prior levels of disease tolerance exhibited by the parental post-epidemic trees. We also conducted population experiments to test prior findings of a correlation between survival and two allozyme alleles (genetic markers). Some half-sib groups and some clonal groups do perform better than other groups when grown in greenhouses and inoculated with the pathogen. This makes a strong case for the presence of genetic resistance. However, no significant correlation between prior parental tolerance under natural disease conditions and seedling tolerance was found. We attribute this finding to a strong environmental component in determining the survival of live oak trees in natural settings. In the study comparing allozyme allele frequencies between pre- and post-epidemic populations, we found no evidence of markers linked to resistance. Further research will be required for the identification of superior live oak selections with reliable oak wilt resistance.

Key words: *Ceratocystis fagacearum*, *Quercus fusiformis*, resistance screening

The oak wilt epidemic in Texas, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has taken a severe toll on urban and rural oak populations (Appel and Maggio 1984, Appel 1995). This epidemic is presently attenuated by applying a variety of management tools aimed at preventing pathogen spread and protecting high risk trees (Appel et al. 2003, Billings, this proceedings). These tools include cautious treatment of firewood, the elimination of inoculum sources and infection courts, trenching to destroy root connections between diseased and healthy trees, and intravascular injection with systemic fungicides. The worth of these measures has been proven and accepted, so they are routinely applied throughout Texas where warranted. They are not, however, infallible and they can sometimes be expensive and environmentally disruptive. Therefore, additional measures are needed to control oak wilt and preserve valuable trees and woodlands.

There are 26 *Quercus* species in Texas. Some are affected by oak wilt more so than others. The most susceptible oaks are members of the deciduous red oak group (genus *Quercus*, sub genus *Erythrobalanus*), such as *Q. buckleyi* and *Q marilandica*. These red oaks do not recover from infection (Figure 1). At the other extreme, deciduous white oaks (genus *Quercus*, sub genus *Leucobalanus*) are extremely resistant to oak wilt and rarely succumb to the disease. They are not, however, the most common component of the central Texas oak savannahs. Semi-deciduous live oaks, *Q. virginiana* and *Q. fusiformis*, are the most common tree in the central Texas rangelands and show variable tolerance to the oak wilt fungus (Appel 1986). The live oaks are classified as white oaks, but exhibit several anatomical characteristics inherent to the red oaks (Muller 1961). Fifteen to twenty percent of infected Texas live oaks survive infection, with the survivors ranging from no crown death to near total mortality (Figs. 2, 3) (Appel et al. 1989). This variable disease response presents a unique opportunity to search for the sources and causes of tolerance, or resistance, to *C. fagacearum* in the native live oak population.

Due to the ability of native live oaks to withstand site disturbances, and their extreme popularity as planted shade trees, live oak comprises a majority of urban trees in Central Texas cities and communities. Given the popularity of live oak in Texas, there is a sizeable potential market for an oak wilt resistant selection. The development of such a resistant selection will not be possible until there is further information on the heritability of resistance and whether live oak survival is the result of one or many resistance genes. Previous researchers presented evidence that the resistance of live oaks was genetic and heritable (Bellamy 1992, Greene and Appel 1994, McDonald et al. 1998). Half-sib groups of live oak seedlings were shown to differ in their resistance (Greene and Appel 1994). A post-epidemic population of live oaks had different allozyme allele frequencies than surrounding pre-epidemic trees by McDonald et al. (1998). Allozymes are different forms of enzymatic proteins that can be visualized by gel electrophoresis to infer different alleles of specific genes (Soltis and Soltis 1989). Differences in allele frequencies between the pre- and post-epidemic populations are evidence for natural selection being exerted on the host by the pathogen, perhaps indicating a shift toward greater resistance in the host.

The objectives of the present research project were designed to expand on the results of both Greene and Appel (1994) and McDonald et al. (1998). This was done by: 1) challenging large numbers of greenhouse-grown half-sib live oak seedling groups with *C. fagacearum* to look for differences in resistance, 2) creating and comparing clonal offspring from post-epidemic trees by screening them for response to challenge by *C. fagacearum*, and 3) evaluating the allozyme profiles of live oaks growing in additional disease centers to test for allele frequency changes in post-epidemic populations. One purpose of the project was to determine if allozymes can be used as markers to recognize trees containing resistance genes. If those efforts are reliable, then they may be selected for breeding and propagating future populations of resistant trees.

MATERIALS AND METHODS

Experiment 1

Seedlings grown for inoculation with *C. fagacearum* were collected from plateau live oaks (*Q. fusiformis*) in 1997 and 1998. One group of live oaks (post-epidemic trees) consisted of survivors in a disease center near Round Rock, TX. The post-epidemic trees exhibited a complete range of crown death (see Fig. 1), so that diseases responses of the artificially infected seedlings could be compared to that of their naturally infected parents. Progeny from these trees were compared to progeny derived from live oaks growing outside of, but adjacent to, the

expanding disease center (pre-epidemic trees). Seedlings were grown for one year in pots with a 4:1 sand/bark mixture and slow release fertilizer and then inoculated with a suspension of *C. fagacearum* conidia (1 x 10^6 spores/ml). The seedlings from each tree were place in random blocks in a shade house and treated uniformly to minimize effects due to environmental variation. Typical disease symptoms were monitored regularly for a year, until disease progress ceased. Responses of seedling groups were compared for each crop using two measures: 1) percentage of group survivors after one year, and 2) percentage of group with less than 25 % crown stem death.

Clonal trees were grown from root spouts collected from post-epidemic tree in 1997. As with the seedlings, the parent trees for clonal sprouts were rated for disease response so that the responses of the clones under artificial inoculation with *C. fagacearum* could be compared to the response of the surviving parent in the infection center. Ramets were cut from live oak root systems, treated with the root stimulating hormone indole-3-butyric acid (Sigma, St. Louis, MO), and planted in "d" pots contain a 4:1 sand bark mixture (Wang and Rouse 1989). The plants were placed in a mist chamber until they grew substantial root systems (approximately 4 months), and then transferred into one gallon pots with the same mix supplemented with a slow release fertilizer. The clones were inoculated after two years growth using identical techniques as those for the seedlings and disease progress followed as previously stated. Clonal group disease responses were measured and compared using average stem death.

Experiment 2

Selected allozymes were analyzed in the leaves of live oaks growing in oak wilt centers at two separate locations in central Texas. One was approximately 10 ha., located in the Balcones Canyonlands Reserve (BCR) in western Travis County near Austin, TX. The other was a rural site north of Lampasas, TX, and was approximately 15 ha. The allozyme profiles of two distinct live oak populations were compared in each of the locations. The first population consisted of post-epidemic, surviving trees located on the interior of the disease center. The second consisted of healthy, pre-epidemic trees on the perimeters of the disease center. Trees from each site were chosen and marked with the limitation that no trees less than 10 m distant from another selected tree was included to avoid clonal individuals. Several leaves (10 - 20 per tree) were collected, transferred to the lab on ice, and processed to provide enzymatic proteins. The leaves were kept refrigerated in the laboratory at 4^o C and processed with 3 days of collection. Standard enzyme extraction, electrophoresis, and gel evaluation procedures were used for allozymes from four polymorphic loci (Stuber et al. 1988). The allozyme frequencies from pre-and post-epidemic populations were compared to determine such population history dynamics as selection, migration, and genetic drift (Ayala 1982, Nei 1978).

Allozymes were analyzed as alleles at individual loci. Therefore, the collection of allozymes at each tree represents its genotype. Allozyme data for all the trees at each site were entered into the software population genetics program "POPGENE-VERSION 1.31" (Yeh and Boyle 1997). The POPGENE program can be used to evaluate allele frequencies, genotype frequencies, genetic diversity, Hardy-Weinberg equilibrium, and a variety of other parameters reflecting the genetic structure and evolutionary background of a population. Details of these analyses will be discussed only in general terms during this presentation.

RESULTS

Experiment 1

Some groups of half-sib seedlings following inoculation had significantly greater average survival after one year than other groups from both the 1998 and 1999 seedling crops. Comparisons among 21 first year (1998) half-sib groups (seedlings surviving for one year after inoculation) resulted in five groups (numbers 1, 2, 3, 12 and 13) that had significantly higher percentages of survival than the five poorest groups (numbers 6, 7, 8, 10 and 15) ($p = 0.05$) (Fig. 4). The best performing seedling group (number 12) had a significantly higher percentage of successful seedlings than the poorest 15 groups. In the second year's crop, 1999, one group (number 13) had a significantly higher percentage of surviving seedlings than 11 out of the other 31 groups with the fewest survivors ($p = 0.05$) (Fig. 5).

When analyzing the proportions of the half sib seedlings with less than 25% stem death, group no. 20 from the 1999 crop was significantly more tolerant than 8 of the 30 other groups. Three of the groups were significantly more tolerant than nine of the least tolerant groups (Fig. 6).

In the comparisons of nine clonal groups with at least three members, one clonal group (number 6) was more tolerant than the three least tolerant groups (Fig. 7). The variances of tolerance within the clonal groups were surprisingly uniform.

The tolerances of half-sib seedling and clonal groups from post-epidemic trees were compared to their parent trees' performances in the field under natural infection by *C. fagacearum*. In general, the seedlings and clones that exhibited increased tolerance tended to have more tolerant parents as estimated by crown survival (Fig. 8). But, the correlation coefficients were all low.

Experiment 2

No specific data comparing the tree allozyme frequencies between pre- and post-epidemic areas of two disease site will be presented in this talk. There were no specific allozymes that had significant pre- to post-epidemic differences in both sites. These results will be discussed in general terms below.

DISCUSSION

These experiments were conducted to find potential sources of resistance to the oak wilt pathogen in native live oaks. Two general approaches were used. Both of these approaches were used in previous, preliminary studies to test for resistance in surviving live oaks growing in oak wilt centers in central Texas (Bellamy 1992, McDonald et al. 1998). The first was to test for unique enzyme profiles (allozymes) in surviving live oak populations to determine whether the pathogen is exerting natural selection for resistant host genotypes. If this was the case, then those survivors are potential sources of selection and breeding efforts to develop superior trees. The second was to collect acorns and root sprouts from those survivors as sources of seedlings and clones, respectively, for inoculation screenings (Green and Appel 1994). Results from the preliminary projects were sufficiently promising to extend them to a broader sample of trees growing over a wider geographic range in the present study.

In the screenings of seedlings, two responses were measured to evaluate potential resistance. The first was average group survival, for which there were differences among groups of seedlings derived from both the 1998 and 1999 acorn crops. Presumably, the best performing seedling groups would reflect some degree of resistance in their parents and point to those trees

as candidates for further analyses. The second response measured in the seedlings was the proportions with less than 25% stem loss. Again, differences among the seedling groups for this criterion indicated there may be variability in resistance to *C. fagacearum* among the parents.

Since live oaks are open pollinated, out-crossing trees, variability in a population of seedlings grown from acorns from a maternal parent for any phenotype such as disease resistance may exist. For this reason, clones from the parental trees were developed to undergo similar screening. There was one clonal group with a significantly greater proportion of stems with less than 25% dieback. This tolerance in one of the groups lends further evidence for a genetic basis for resistance to oak wilt in the surviving native live oaks.

The methods used in the present study also have been used for identifying sources of resistance to a variety of diseases in other tree species. For example, clones have proven effective in testing for disease resistance in other species, such as elms for Dutch elm disease (Solla et al. 2005). In addition to the genetic basis for resistance, Solla et al. (2005) mentioned a wide number of other factors as being influential in the disease response. These included time of inoculation, environmental conditions, and even height of the inoculated elm saplings. These factors were probably influential in the present study on oak seedlings and saplings, adding to the variability in disease response and perhaps confounding the discovery of a clearly resistant selection. Nonetheless, the results are sufficiently encouraging and some useful materials have been found for continued propagation and testing.

An additional, useful measure of heritability for resistance is the relationship between the responses of progeny to artificial inoculation compared to the performance of the parents under natural infection in the field. Similar considerations are being made for other tree diseases, such as efforts to find disease resistance in native butternut (*Juglans cinerea*) to the exotic canker causing pathogen *Sirococcus clavigignenti-juglandacearum* (Michler et al. 2005). No significant trends were detected when the response of seedlings were compared to those of their parents, indicating a parent's prior performance cannot be used to confidently predict the tolerance or resistance of the offspring toward the pathogen.

The two allozyme alleles that McDonald et al. (1998) found associated with survival were tested in this study and were not associated with survival in either disease center. We found no decrease in genetic diversity as was reported in that previous study. We did find that all live oak populations were in Hardy-Weinberg equilibrium which shows that these populations are maintaining genetic diversity through sexual reproduction instead of being only large populations of a few clones. Allozyme profiles of individual trees and GPS mapping did allow us to find several clonally propagated motts within the larger populations. The allozyme data also was used to show that the two disease sites, although separated by approximately 80 km, had nearly identical allele frequencies. This finding was evidence that gene flow is widespread and that natural populations of live oaks through out the Edward's Plateau in central Texas should be expected to share similar genetic profiles.

CONCLUSIONS

A genetic basis for tolerance to oak wilt caused by *C. fagacearum* does exist in live oaks, and this tolerance is heritable. However, the level of crown loss in post-epidemic trees is a poor predictor of how offspring from those trees will perform when challenged with the fungus. Environment or chance plays a substantial role in the outcome of this disease in live oaks. Individual clonal groups show a more normal variation of response to the fungus. Two clonal groups have been found that showed consistent tolerance and may indicate a source of tolerant

trees. The allozyme markers that were studied in this project are not useful marker to identify resistant live oaks. Future research should take advantage of artificially-created live oak clones and revisit environmental effects upon the disease process.

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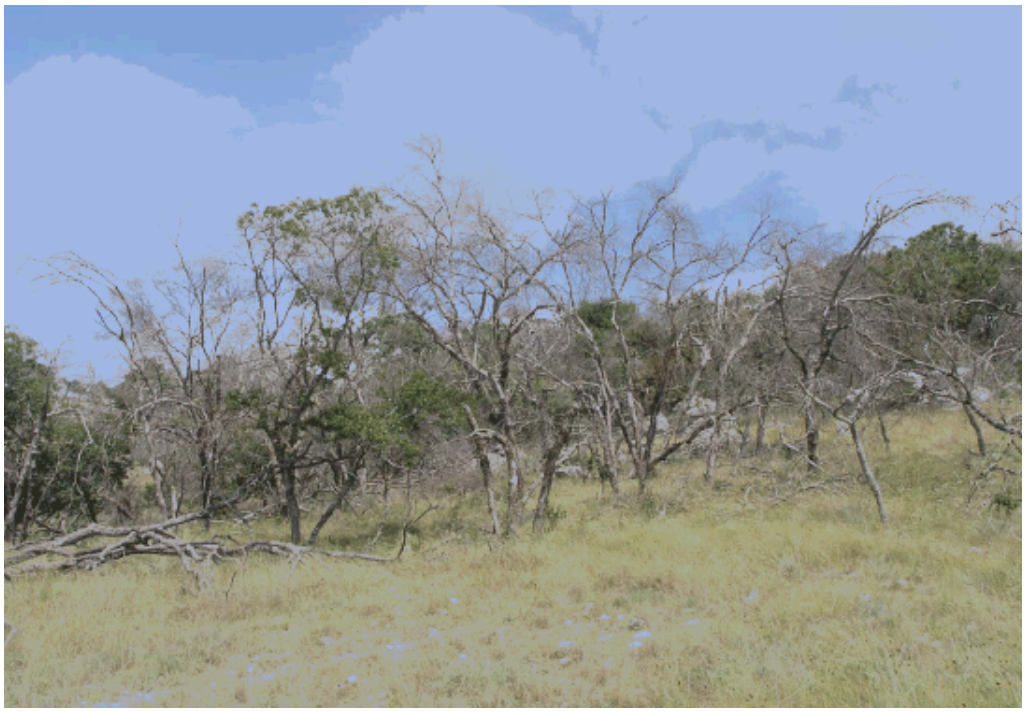

Figure 1. Red oaks in central Texas killed by *Ceratocystis fagacearum* exhibiting no survival.

Figure 2. Live oak within an oak wilt center in central Texas exhibiting partial survival following infection by *Ceratocystis fagacearum*.

Figure 3. Variable survival rates of live oaks within a typical central Texas oak wilt center.

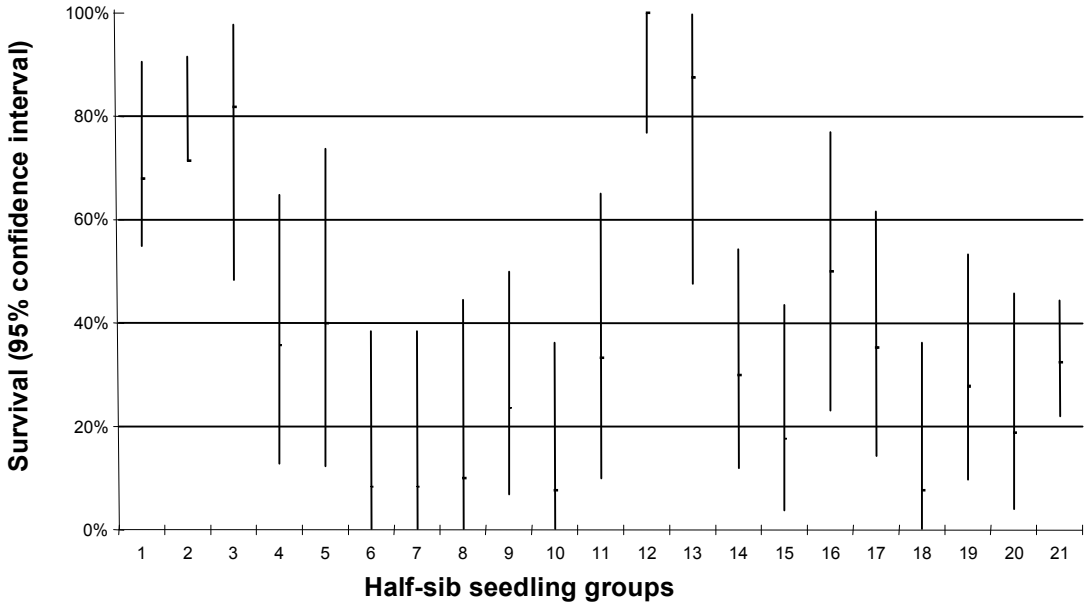

Figure 4. Comparison of survival (% alive) of 21 seedling groups grown from the 1998 acorn crop from live oaks in central Texas one year after inoculation with *Ceratocystis fagacearum*.

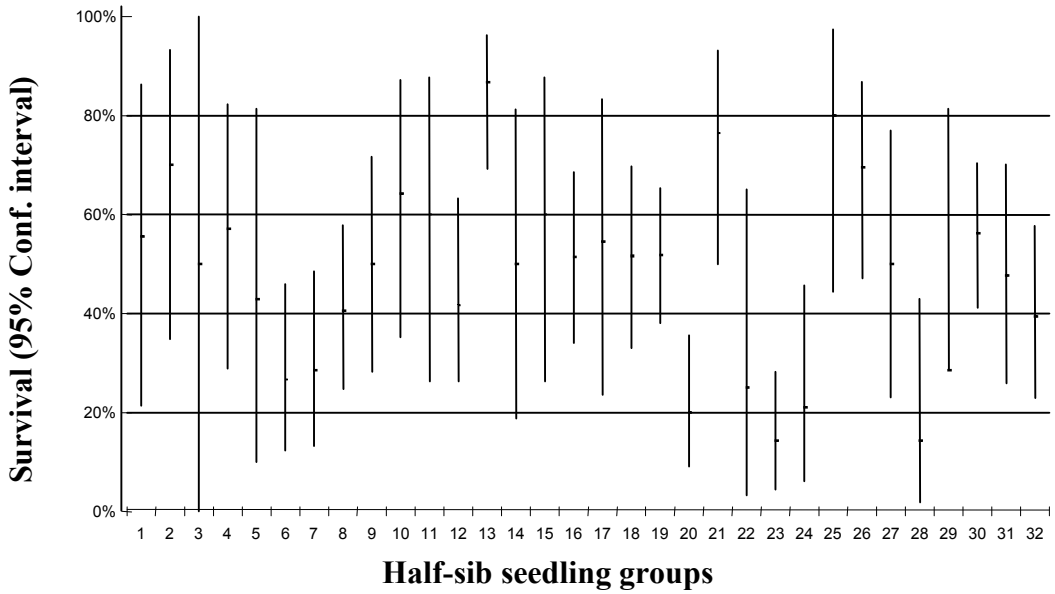

Figure 5. Comparison of survival of 32 seedling groups grown from the 1999 acorn crop from live oaks in central Texas one year after inoculation with the *Ceratocystis fagacearum*.

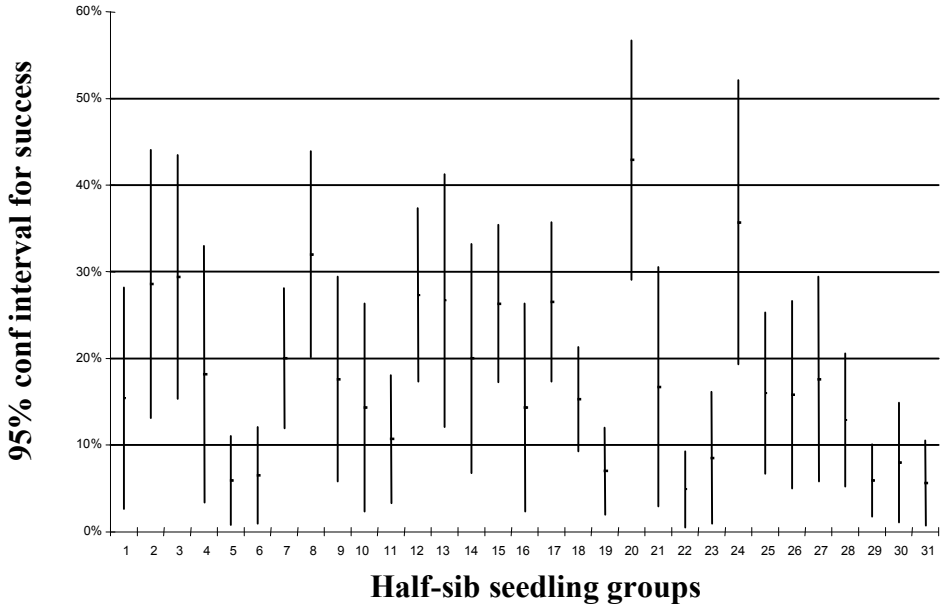

Figure 6. Tolerance, as defined by less than 25% stem loss, in groups of half sib seedlings one year following inoculation with *Ceratocystis fagacearum*.

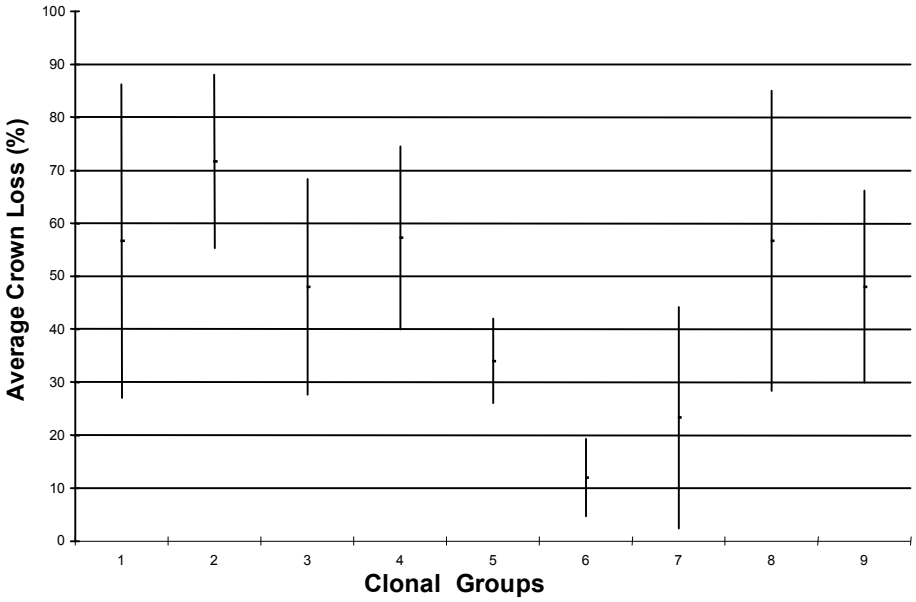

Figure 7. Average crown loss for groups of live oak clones inoculated with *Ceratocystis fagacearum.*

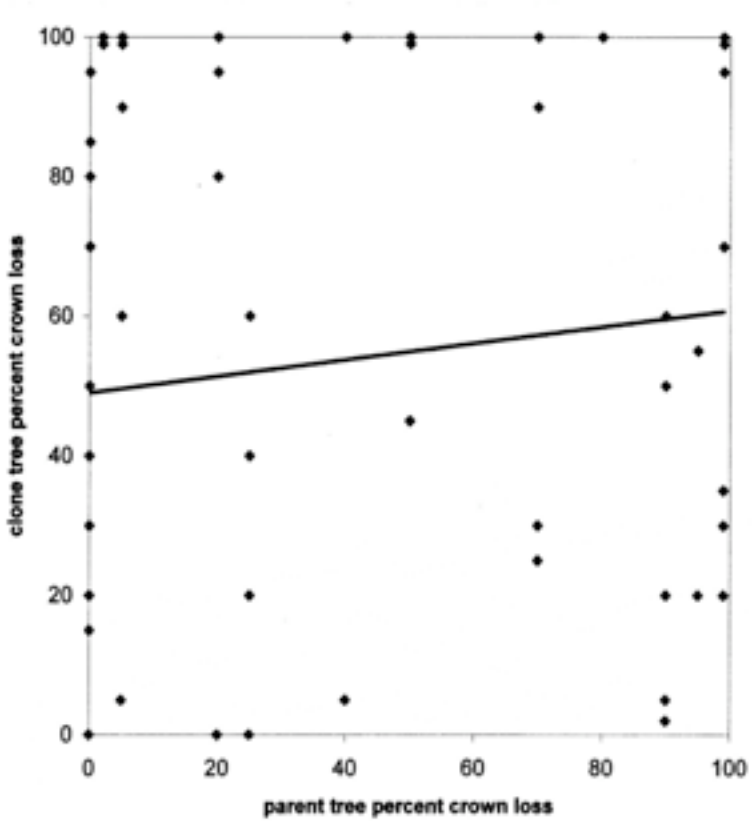

Figure 8. Correlation between the proportions (percent) of crown loss in clonal saplings artificially inoculated with*C. fagacearum* and the crown loss of their naturally-infected parents.

THE POTENTIAL OF TRENCH INSERTS FOR OAK WILT SUPPRESSION

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ABSTRACT

Trench inserts are physical barriers used to control root transmission of *Ceratocystis fagacearum* which provide a significant new strategy and technology for oak wilt suppression in the U.S. This cultural control method has been shown experimentally to significantly extend the effective life and utility of trenches in Texas. The utilization of trench inserts also has increased the effectiveness of trenches as physical barriers to root transmission. Water-permeable trench inserts are more effective barriers than trenches alone because they prevent new root graft formation in trench-backfill soil indefinitely. Trench inserts may provide greater insurance against future trench breakouts in backup trenches when original trenches fail. Water-impermeable trench inserts are not as effective because, in some cases, they tend to direct root growth around (usually above) the insert when inserts are buried too deeply. Trench inserts may be installed at a fraction of the costs of primary trenches and may not significantly increase total trenching costs. The use of trench inserts could potentially save millions of dollars through protection of uninfected trees, avoidance of tree removal costs, and reductions in property value depreciations for Texas landowners. This technology is equally applicable in other areas of the U.S. affected by this disease. Some potential problems associated with the installation of trench inserts are discussed.

Key words: *Ceratocystis fagacearum*, direct control, root barriers

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is probably the most destructive disease of oak species in the United States (Gibbs and French 1980, Appel 1995, Tainter 1995, Wilson 2001). The disease annually kills numerous oaks throughout the eastern half of the country, particularly those in the red/black oak group (subgenus *Quercus* section *Lobatae*) due to greater susceptibility to the disease and differences in physiology and microscopic anatomy of sapwood (Tillson and Muller 1942, Nixon 1993, Tainter 1995, Wilson, Lester and Oberle 2005). The semievergreen live oaks, including plateau live oak (*Q. fusiformis* = *Q. virginiana* var. *fusiformis*) and coastal live oak (*Q. virginiana*), are considered the most valuable woodland and urban tree species in central Texas (Appel et al. 1986, Martin, Maggio and Appel 1989). Although live oaks are intermediate in susceptibility to oak wilt, they are the most seriously affected oaks in terms of disease incidence and rate of spread. The natural growth tendencies of live oaks to form root sprouts from mother trees, giving rise to large clusters of clonal trees (motts) with extensive root grafts and interconnected common root systems, increase the predisposition of live oaks to root transmission (Muller 1951, Appel, Anderson and Lewis 1986, Davies 1992, Wilson 1995). Oak wilt disease incidence is often high in live oaks because of these growth-form predispositions, the formation of extensive shallow root systems, and the abundance and high density of live oaks in both urban and rural forest stands of central Texas.

Most live oaks defoliate and die within six months after the initial appearance of oak wilt symptoms. Consequently, there is very little time to initiate effective disease-control measures to save individual infected trees after symptoms first appear. Any tactics developed to halt disease spread must consider that disease development often occurs rapidly and the oak wilt fungus can move up to 50 m or more per year through grafted root systems at the edge of expanding oak wilt infections centers.

Trenching to cut root connections between healthy and diseased trees continues to be the principal means of controlling the spread of the oak wilt fungus through root transmission in the U.S. (Himelick and Fox 1961, Cameron and Billings 1995, Gehring 1995, Billings et al. 2001, Haugen et al., this proceedings). Trenching has been a particularly important tool for dealing with the disease in highly-valued live oak stands because root grafts and extensively interconnected root systems allow the disease to spread rapidly by root transmission. The Texas Oak Wilt Suppression Project (TOWSP), administered by the Texas Forest Service since 1988, has installed over 3.4 million linear feet of trench to combat the disease in 2,466 oak wilt infection centers found within 61 of 254 Texas counties (Billings, this proceedings). However, trenching is not totally effective in containing the disease, partly due to new root connections that form across the trench and allow disease centers to continue to expand beyond trench barriers. TOWSP post-suppression evaluations showed that about 33% of trenches installed prior to 1995 had at least one breakout (infected trees beyond the trench), although seldom is the entire trench a failure (Gehring 1995).

A 7-year study was initiated in 1993 to look at the feasibility of improving the effectiveness and longevity of trenches using trench inserts (Wilson and Lester 2002). A significant finding in this study was that small oak feeder roots, formed from roots severed by trenching, grew into the loose backfill soil within trenches allowing new root graft connections to form across the trench. The results showed favorable indications that trench inserts could provide significant improvements in the performance of trenches by preventing the formation of these new root grafts that allow inoculum (conidia and hyphal fragments) of the oak wilt fungus to move beyond the trench. Preliminary research demonstrated the efficacy of trench inserts in stopping the expansion of oak wilt infection centers beyond the trench up to six years after trenching (Wilson and Lester 1996a-c).

This paper explains why improvements in trenching technologies are needed to more effectively control root transmission of *C. fagacearum*, how trench inserts improve trench effectiveness and longevity, what additional expenses are associated with utilizing trench inserts, the comparative performance of different types of trench inserts, and some potential problems involved in the installation of trench inserts for oak wilt control. Recommendations also are provided that explain ways to more effectively and efficiently implement the use of trench inserts within existing oak wilt suppression programs.

TRENCHING EFFECTS ON SOIL STRUCTURE AND ROOT INTERATIONS

The first essential information needed for improving trenching technologies for oak wilt control is to determine why trenches fail or why trench breakouts occur. The principal reasons why trench breakouts occur usually vary with time after trench installation. Breakouts that occur within two years after trenching usually result from placing the trench too close to the infection center or not trenching deeply enough to sever all roots. Breakouts that occur after 2-3 years are likely the result of root regrafting across the trench. Trenching causes changes in the physical properties of soil structure and subsequent root interactions that occur in trench backfill soil after

trenching. These effects of trenching on soil structure and oak-root interactions set into motion a dynamic process that ultimately increases the chances for trench breakouts over time following trenching.

PROBLEMS WITH TRENCHING

One of the biggest problems associated with the mechanical cutting of trenches for oak wilt control involves the effects of trenching on soil structure and characteristics within the trench. Trenching creates a soil environment highly favorable for root growth. Farmers till the soil in their fields before planting for this very reason. Tilling the soil reduces the bulk density of soil by breaking up soil aggregates into smaller particles, rendering the soil friable and more favorable for root growth, penetration by rainfall, and infiltration by fertilizers and nutrients. For the same reason, no-till cultivation has been adopted as a farming practice in many areas of the U.S. to reduce soil erosion and weed growth stimulated by the loosening of soil structure that facilitates root growth of competitive weed plants. Trenching has the same effects on soil structure as tilling the soil. Backfill soil within the trench is loosened significantly (bulk density 1.22 g/cm^3) relative to the compacted surrounding soil (bulk density 1.62 g/cm^3) (Backhaus 2005).

There are several consequences that result from these changes in soil structure due to trenching. Roots from the surrounding compacted soil seek to grow in this loose backfill soil because it provides the path of least resistance for root growth, expansion, and branching. Consequently, roots proliferate and penetrate into this loose trench backfill soil much more rapidly than in the adjacent, compacted soil on either side of the trench. The loosened backfill soil within newly-cut trenches also is a natural sink for water and nutrient flow into the ground, again because it is the path of least resistance. Water and dissolved nutrients tend to collect and accumulate within the loosened trench soil, further enhancing and stimulating new root growth from roots adjacent to the trench. The natural influx of tree root growth into this more favorable trench soil is promoted by all of the improvements in soil conditions that result from the trenching process.

The cutting of oak roots by trenching induces the formation of fine (<5 mm-diameter), feeder roots in trench soil due to the loss of apical dominance. This same phenomenon occurs when apical meristems are cut in the upper parts of the tree resulting in the loss of apical dominance and the production of sucker sprouts on trunks and lateral epicormic branches. The production of lateral roots, due to the loss of apical root dominance in roots severed by trenching, leads to the proliferation of fine feeder roots in the loose backfill soil of newly-cut trenches. These feeder roots begin forming within a few inches of the severed ends of roots as soon as soil moisture becomes available. Trenches are generally backfilled immediately during the trenching process, allowing new root growth to begin forming usually after the next rain event. Most feeder roots are found within the top 18 inches (46 cm) of the soil surface where soil moisture is most available following a rain. Feeder roots also form from the cut ends of deeper roots severed by trenching, up to 6 feet (1.8 m) or more below the soil surface depending on soil depth.

The very high tendency of Texas live oaks to form root grafts and common root systems allows these species to utilize the ideal conditions within newly-cut trenches to initiate the formation of new fine feeder roots that readily graft with roots of other live oaks with which they come in contact. Because live oaks on both sides of the trench have roots severed by trenching, these trees send out an abundance of new feeder roots into trench soil creating conditions very favorable for the formation of new root grafts across the trench. It is for this reason that the

TOWSP recommends uprooting all trees and disrupting or extracting the root system inside the trench in rural areas.

TRENCH BREAKOUTS – WHY THEY OCCUR

The majority (60.1 %) of oak wilt breakouts from TOWSP trenches have occurred during the first two years after trench installation (Table 1), due to factors other than regrowth of roots across the trench (Gehring 1995). The rate of new trench breakouts decreases rapidly over time after two years resulting in negative slope curves for breakouts beyond two years after trenching. Nevertheless, up to 40% or more of all trench breakouts occur after two years following trenching. Even though trench breakout rates continue to decrease after two years, breakouts have been recorded to occur up to fifteen years or more after trench installation, far beyond the time normally expected for *C. fagacearum*-inoculum to move through existing root grafts across the trench. The expanding edges of oak wilt infection centers are known to move up to 80 feet (25 m) or more per year in Texas live oaks. Because trenches normally are installed within a 100-feet (30 m) buffer zone beyond the advancing front of the infection center, it usually takes a maximum of two or three years for *C. fagacearum*-inoculum to move through any preexisting root connections that were not severed by the trench.

Breakouts that occur after two years are increasingly more likely to be due to transmission of the fungus through new root grafts that formed after trenching (Table 2). The delayed timing of later breakouts is due to the time required for new root grafts to form and provide a route for inoculum in the roots to pass beyond the trench barrier. Most breakouts occurring within the first two years after trenching have been attributed to inoculum passing through pre-existing root grafts either due to 1) insufficient trench depth, 2) insufficient buffer distance set up between the visible (symptomatic) advancing edge of the infection center and the positions selected for trench placement, or 3) a discontinuous trench (Gehring 1995).

Trenches that are not cut to sufficient depth allow the fungus to pass through roots that were not severed under the trench. This occurrence is common where the soil depth to bedrock in localized areas is greater than the depth normally encountered in that area or when soil depth is greater than the depth recommended by suppression-operation criteria. Cutting the trench too close to the infection center, without leaving a sufficient buffer zone, can also be a problem. Trenches placed too closed to diseased trees fail because the fungus has already moved through roots by the time the trench is installed. Discontinuous trenches, often caused by the need to avoid buried utility lines, provides opportunities for inoculum to pass through gaps in the trench where roots were not severed. Obviously, the rates of trench breakouts, due to discontinuous trenches, tend to increase in urban areas.

The TOWSP does not cost-share continuous trenches unless the utility lines have been installed at least four feet deep with the past four years. Even though most trench breakouts occurring after two years following trenching occur due to new root graft formation, some breakouts occasionally may occur due to movement of *C. fagacearum*-inoculum across the trench by insects or other vectors (Table 2). The movement of red oak firewood from infected trees within infection centers to areas outside of the trench also may explain some jumps or gaps in infection patterns in the vicinity of trenches. There is also the possibility that unknown root-feeding or stem-feeding insect vectors may be carrying inoculum of *C. fagacearum* across trench barriers.

Problems of trenching associated with improper trench depth and buffer distance can be largely solved by increasing trench depth when possible and increasing the buffer zones used for

determining trench placement. These adjustments have been made several times in the operational criteria used for trench installation in TOWSP operations. Unfortunately, modifications in trench designs and placements do not solve the problem of breakouts due to other causes, particularly the formation of new root grafts in the loose trench backfill soil. Trenches alone are not permanent by design. They cease to become a barrier as soon as new root grafts form across the trench. The very high tendency of live oaks to form root grafts, coupled with the greater incidence of root growth and root graft formation in trench backfill soil, increasingly favors the ability of *C. fagacearum*-inoculum to eventually move through a new root graft connection to the other side of the trench over time.

The actual likelihood that new root grafts will form in the trench backfill soil in any one location is dependent on a number of factors, including weather and rainfall patterns, the presence and density of trees on both sides of the trench, the amount of inoculum-pressure put on the trench (determined by the size of the infection center being contained), the rate of movement of the infection front, the depth of the soil and/or trench, soil texture and fertility, and slope of the terrain. However, the rate of new root graft formation in trench soil is probably most determined by available soil moisture in the trench, the density of live oaks on both sides of the trench, and the depth of the soil in the trench. These three factors probably have the greatest effect on feeder-root density, and thus determine the likelihood that new root grafts will form across the trench.

An alternative explanation has been proposed to explain breakouts that occur beyond two years after trenching. This explanation suggests that these breakouts may be due to insufficient trench depth instead of the formation of new root grafts in the trench soil. The theory implies that roots occur under the trench by which the oak wilt fungus eventually passes, but that the movement of *C. fagacearum*-inoculum in the roots is delayed by insufficient rainfall, inadequate tree transpiration, or other factors that somehow slow down the movement of inoculum in the root system and delay trench breakouts. The problem with this explanation is that the movement of water carrying *C. fagacearum*-inoculum in the transpiration stream of roots through root grafts is controlled mostly by the transpiration of healthy trees outside of the trench that pulls water from the root systems of diseased trees inside of the trench.

Transpiration rates in the stems of oak wilt-infected trees are slowed due to vascular plugging by the fungus. Consequently, transpiration rates are higher in healthy trees that have no vascular plugging. This is the reason why transpiration water tends to flow mostly away from diseased trees (at the expanding edge of the infection center) toward uninfected healthy trees (outside of the trench) that still have substantial transpiration occurring. This is the only reasonable explanation to account for the often rapid rates (75-150 feet or 23-46 m per year) of expansion observed in Texas oak wilt infection centers. Given this strong outflow of transpiration water through preexisting root grafts, that were not severed between diseased trees at the edge of infection centers and healthy trees outside of the trench, there should be a very low probability that *C. fagacearum*-inoculum would not pass through one of these preexisting root grafts within the first two years after trench installation.

The movement of transpiration water through root grafts to healthy trees can be slowed by drought conditions. However, if there is a root connection path (root graft) around or under the trench for inoculum to pass through, it should occur within two years because movement of transpiration water is analogous to water running downhill by gravity. Healthy trees cannot survive for very long without transpiration. Anything being carried by the water, whether it is dissolved nutrients or *C. fagacearum*-inoculum, is moved in the transpiration stream and taken

up by the roots of healthy trees outside of the trench. Therefore, it is reasonable to assume that breakouts that occur beyond two years after trenching are due to newly formed root grafts in the trench because all old preexisting root connections were effectively severed by trenching. In the absence of an extended drought, the delay in trench breakouts beyond two years is increasingly more likely caused by the delay in formation of new root-connection paths across the trench by which inoculum can be carried to healthy trees outside of the trench. The only other appreciable factor that could lead to a delay in trench breakouts beyond two years after trenching is placement of the trench significantly more than 100 feet beyond the infection center. In this case, it would take longer for the fungus to traverse the greater distance through connected root systems to challenge the trench. The greater time required before the trench is challenged provides more time for new root grafts to form across the trench, increasing the chances for trench breakouts when inoculum of the fungus finally arrives at the trench. More evidence for trench breakouts due to new root graft formation is provided under the section Trenching Results in a Metropolitan Area.

EXPERIMENTAL EVIDENCE FOR TRENCH INSERT EFFECTIVENESS

A 7-year USDA-Forest Service research study was initiated in 1993 near Austin, TX to evaluate the efficacy for using trench inserts as a new cultural control method for the management of oak wilt in Texas (Wilson and Lester 2002). This study addressed the need to reduce the incidence of trench breakouts that occur beyond the first two years after trench installation due to causes other than improper trench placement or insufficient depth. The failure of primary trenches to prevent root transmission of *C. fagacearum* usually requires the installation of expensive backup trenches to attempt to contain further expansion of these unchecked oak wilt infection centers.

Sometimes even backup trenches fail, leading to additional costly trench breakouts that may be too large and expensive to contain. The larger an oak wilt infection center becomes, the more expensive it is to contain because approved trenching projects require installation of a suppression trench completely around the infection center. One of the key objectives of this 7-year trenching study was to determine why trench breakouts occur beyond the first two years after trenching when trench breakouts are normally expected to occur. A better understanding of trench breakouts and how they occur was needed in order to find new ways to extend trench utility and effectiveness beyond two years, and thus avoid costly trench breakouts.

The effects of trenching on soil structure in the trench and resulting root growth in trench backfill soil following trenching was evaluated with an experimental trench having different treatment segments set up along its length. Treatment segments consisted of four types of trench inserts, trench alone, and no trench controls to determine whether trench breakouts were affected by the presence or absence of trench inserts. The experimental hypothesis being tested was whether trench inserts affect the number and timing of trench breakouts relative to trench alone or no trench controls. If a difference in timing of breakouts could be detected between treatments, this would indicate that there must be a fundamental difference in the process or timing of root transmission events that explains the common delays in trench breakouts that are often observed with trenching treatments compared with no-trench controls.

PERFORMANCE OF WATER-PERMEABLE VS. WATER-IMPERMEABLE TRENCH INSERTS

The efficacy of trench inserts in preventing root transmission of *C. fagacearum* was tested in order to determine the effects of different types of trench inserts on trench performance. Four types of trench inserts, consisting of two water-permeable materials and two water-impermeable materials, were tested and compared to trenches alone and no trenching segments. These six treatments each were replicated three times in a random sequence along the full length of a continuous 0.75-mile (1.2 km) trench located 100 feet (30 m) beyond the expanding edge of a large oak wilt infection center.

The two water-permeable inserts consisted of 4 oz. (113 gm) (1×) Typar, a spun polypropylene landscape fabric, and Biobarrier which contains the same fabric as Typar, but also contains trifluralin-impregnated 10-mm diameter, controlled-release hemispherical pellets (54% polyethylene, 18% carbon black, and 28% trifluralin by weight) bonded to polypropylene fabric with uniform 3.8-cm spacing or 688 pellets per square meter (Reemay Inc., Old Hickory, TN). The water-impermeable insert materials consisted of polyethylene Rufco Geomembrane liners (Raven Industries, Springfield, OH) of two thicknesses (20 and 30 mil), namely Rufco 2000B, and Rufco 3000B, respectively. Trench inserts were placed into trenches in 15.2 or 30.5 m lengths, mounted with 15 cm steel or aluminum pins to the wall of the trench on the side closest to the infection center, and additionally supported by backfilling the trench with soil removed during construction of the trench, followed by leveling with a backhoe scoop blade (see Wilson and Lester 2002).

The occurrence of new trench breakouts of oak wilt disease by year for six years following trenching provides a comparison of performance of the six treatments (Table 3). The study was established during an extended period of drought which caused trench breakouts to be somewhat delayed as a result of reduced transpiration in test trees. The first appearance of oak wilt beyond a no-trench segment occurred the second year after trenching. A second appearance of oak wilt beyond a no-trench segment occurred the third year. One trench segment of Geomembrane 20 (Geo 20) also had a disease breakout the third year. Two trench breakouts occurred in Geo 20 segments the fourth year after trenching. Excavations of Geo 20 trench breakout segments indicated that small roots had grown across the trench in the soil above the trench inserts. Apparently, the Geo 20 trench inserts were buried too deeply in these segments allowing new root grafts to form between feeder roots across the trench above the insert material that resulted in trench breakouts.

One trench-only segment had a trench breakout the fourth year after trenching, also likely due to new root grafts forming across the trench in the loose backfill soil. The last trench breakout recorded in the study occurred the fifth year after trenching in the no-trench segment. However, none of the nine trench segments containing Typar, Biobarrier, or Geo 30 inserts had breakouts of oak wilt during the entire six years of this test. Based on these limited number of treatment replications, the water-permeable inserts (Typar and Biobarrier) appeared most effective in preventing the formation of new root grafts across the trench in trench backfill soil up to six years after trenching

The breakouts occurring in the water impermeable Geo 20 segments may have occurred as a result of the diversion of root growth around the trench insert (toward the surface) after roots came in contact with the material. This diversion of root growth tends to occur with water impermeable materials because the roots cannot obtain moisture through the insert material and continue to grow whereas the presence of moisture through the barrier (as in the water permeable inserts) causes the roots to branch dichotomously against the barrier instead of continuing to elongate in search for moisture. Consequently, water-permeable inserts generally are more

effective barriers to root graft formation because they do not cause significant diversion of root growth after contact with the material.

TRENCHING RESULTS IN A METROPOLITAN AREA

The causes of oak wilt trench breakouts in urban and suburban trenching projects that did not install trench inserts were investigated further to see if more information could be deduced from the results of trenching at different depths using conventional trenching methods recommended by the TOWSP. A series of 24 trenches installed over a 17-year period (1989-2006) by the city of Lakeway, Texas was selected as a model system for this investigation. These trenching projects were placed into two categories, based on trench depth, for the purpose of data interpretation: 1) ten 30 to 36"-deep trenches installed from 1989-1999; and 2) fourteen 39 to 48"-deep trenches installed from 1997-2006. The relative effectiveness of trenches, within these two trench-depth categories, in preventing trench breakouts over time following trenching, is summarized based on observations as of May 2007 (Table 4).

Among trenches in the 30-36" category, 10% held up with no trench breakouts up to 8 years after trenching, 50% had trench breakouts within the first two years after trench installation, and 40% exhibited breakouts 5-14 years after trenching. By comparison, trenches in the 39 to 48" category had significantly higher percentage (42.9%) of trenches without breakouts up to five years after trenching than trenches in the shallower category over the same time interval. A significantly lower percentage (21.4%) of deeper trenches had breakouts within the first 2.5 years than the shallower trenches. However, there was no significant difference in the percentage of trench breakouts between shallow vs. deep trenches that occurred three or more years following trench installation. This surprising discovery indicates that increasing trench depth provides little benefit in reducing trench breakouts that occur more than two years after trenching when trench inserts are not used.

The absence of a difference in breakouts with trenching depth after two years suggests that there is a fundamental different event that is occurring after three years and beyond that is the cause of breakouts which is different from those occurring within the first two years after trenching as indicated by Gehring (1995). The most probable explanation for this result is the formation of new root grafts across the trench which can occur regardless of trench depth in the trench backfill soil. Because most feeder roots are found in the top 18 inches below the soil surface, this is the area of the soil profile where most new root grafts likely form after trenching. Nevertheless, feeder roots are also found at the ends of deep roots that can form new root grafts across the trench following the installation of deeper trenches. Even though deeper trenches appear to be a benefit primarily within the first two years after trenching (when most breakouts occur), the installation of deeper trenches probably does provide greater long-term protection against trench breakouts when trench inserts are utilized. The greater trench depths with inserts will provide assurance that the deeper feeder roots will not form new root grafts across the trench beyond the second year after trenching.

Additional evidence to support the assertion that greater trench depth is an important factor in improving the long-term effectiveness of trenches with trench inserts is forthcoming from TOWSP trenches installed since 2004 by Texas landowners under the direction of TFS employees in Bandera and Kerr Counties. So far, over 20,000 linear feet of trench have been installed with the Typar insert material in trenches ranging from 5-14 feet (1.5-4.3 m). In the past three years, none of these trenches with Typar inserts have had trench breakouts of oak wilt. However, long-term observations will be required to fully assess the efficacy of trench inserts.

As the numbers and linear feet of trenches with Typar inserts expands, the TOWSP personnel will be able to better evaluate the effectiveness of these water-permeable inserts in actual oak wilt suppression trenches.

COSTS ASSOCIATED WITH TRENCH INSERTS

Any assessment of the efficacy of a new disease-control method must not only consider the effectiveness of the control method, but also the necessary additional costs that would be required to implement and utilize the new method. A full-scale economic assessment is not suggested here, but rather an examination of the probable costs of materials alone in the absence of variable costs such as additional labor and equipment modifications needed for implementation. The approximate costs of trench insert materials currently available for oak wilt suppression are provided and compared as a percentage of the average TOWSP trenching costs in urban ($16.85/linear foot) and suburban ($3.47/linear foot) environments since 2004 without trench inserts (Table 5). Trenching costs can be much higher in some cases. These estimated material costs are based on trenches that are at least 48 inches deep (with inserts at least 48 inches wide) at current prices.

The Typar and Geomembrane materials cost $1.20 or less per linear foot and represent only 2-7% of urban trenching costs and 11-34% of suburban trenching. The Biobarrier products with the slow-release trifluralin herbicide nodules cost $7-9 per linear foot representing 41-53% of urban trenching costs and twice as much as suburban trenching costs, significantly more than the other materials. Even though Biobarrier is more expensive than Typar alone, it does provide the additional protection of stopping root growth and elongation, precluding root contact with this material. Thus, Biobarrier provides a chemical barrier in addition to the physical barrier provided by the Typar fabric of which it is composed.

Theoretically, this integrated control with Biobarrier, utilizing two different strategies (chemical and physical), should be more effective than a physical barrier alone. As a statement of confidence, the manufacturer (Reemay Inc., Old Hickory TN 37138) guarantees this material will prevent root punctures up to 15 years. Another advantage is that trifluralin herbicide is water insoluble and will not contaminate groundwater aquifers. All of these trench insert materials have very similar puncture strengths, but the Typar material was the lightest (4 oz. per linear foot) among those tested, and performed as well as the Biobarrier products in experimental tests. The water permeable Typar material also was the cheapest material (only 40¢/linear foot) that was tested experimentally. As implied earlier, these costs do not include the additional labor costs required for installing the trench inserts or the additional costs associated with backfilling the trench by equipment other than the original trencher.

A more comprehensive list of commercially-available Typar products shows that this geotextile material comes in a wide range of weights, puncture strengths, and widths (Table 6). The landscape-grade Typar materials are most appropriate for trench-insert applications in oak wilt suppression, particularly Typar 3401 which comes in 48 and 60 inch widths, the most common trench depths used in oak wilt suppression. For deeper soils, trenches cut greater that 60 inches deep should utilize the Typar 3341 or a similar product because it is available at a width of 151 inches and may be cut down to appropriate widths at the factory if requested. Typar products in the 3500 and higher series are generally designed for more heavy-duty applications such as for road and storm drain construction, and are probably overkill for most oak wilt suppression applications, although some oak species under certain situations may be able to exert sufficient root puncture pressure to warrant use of these stronger materials.

PRECAUTIONS IN SELECTING AND USING TRENCH INSERTS

New roots forming from severed roots after trenching can grow both over and under the insert material, especially with water impermeable trench inserts which have a tendency to direct root growth along the face of the material and around the barrier. By contrast, water permeable inserts tend to cause these new roots to branch and form finer roots that stop elongating, once they come in contact with these inserts, because the roots are able to obtain moisture through the material. Consequently, water-permeable inserts tend to perform better than water-impermeable inserts. Trench inserts should be installed carefully so that the top edge of the material is even with the soil surface to prevent root growth over the top of the buried insert. Some insert materials such as polypropylene geotextile fabrics breakdown readily in the sunlight over a relatively short time. Consequently, materials such as Typar must be fully covered by soil to protect them against sunlight. Thus, it is equally important to not bury trench inserts so shallowly that the material is not covered and subject to degradation by sunlight or exposure to the elements, but extends as deeply as possible in the trench.

There are two major types of geotextile fabrics, spun and woven, that are available for landscape applications. Spun fabrics have greater stretching capacity, are more flexible, and have greater water permeability due to greater numbers of micropores. However, woven fabrics have greater total puncture strength (50%) than spun fabrics, but do not stretch appreciably and are less flexible than spun fabrics. Woven landscape fabrics have holes between the weave that sometime allow fine roots to penetrate. Thus, spun fabrics generally are more durable due to flexibility, and have are more effective in preventing root penetrations than woven landscape fabrics.

The utilization of untested trench insert materials should be considered with much caution because they may potentially lead to trench failures, poor results, and greater tree mortality than would have occurred without any trench inserts. Failures associated with the use of untested insert materials can reduce landowner confidence in trench inserts as an oak wilt suppression tool. Previously, untested landscape fabrics have been used that proved to be ineffective because they decomposed in the soil (lacked durability), had little root-puncture resistance, or were water-impermeable.

DIFFICULTIES IN APPLYING TRENCH INSERTS

The current foremost obstacle to utilizing trench inserts for the suppression of oak wilt root transmission is the fact that most rock saws commonly used in central Texas for trenching are designed to back fill the trench immediately after it is cut, precluding the installation of trench inserts. The trench must be left open temporarily after trenching to permit the installation of inserts along the wall of the trench. The cost of retrofitting a typical rock saw to leave the trench open can cost thousands of dollars. Thus, to be efficient, trenchers designed to leave the trench open must be used and dedicated for this purpose. Backhoes that are often used to dig trenches deeper than five feet favor the installation of trench inserts because the trench can be left open as long as needed.

Ultimately, the best solution would be to design a trencher that not only cuts the trench and leaves it open, but also contains a vertical post on the back for dispensing the insert material into the trench so that the insert can be secured to the wall of the trench, and the trenched backfilled immediately by a bulldozer with a grading blade that follows behind. The two pieces of equipment working together may be able to get the job done faster and perhaps more cheaply due to less rental time. If a bulldozer is used to dig the trench, it should also be used to refill it.

The additional labor, equipment, and fabric costs associated with the installation of trench inserts may be sufficiently large to impact the decision to install trench inserts. For example, the rising cost of urban trenching may, in some cases, prohibit the addition of any further expenses because additional costs may exceed the available budget of a trenching project. Other additional trenching costs may include the necessary removal of soil from trench sections that collapse or cave-in before the inserts can be installed. Many of these additional expenses sometimes can be handled more cheaply by landowners that have access to the proper equipment, such as a backhoe to cut the trench, and have the skill to operate rented or borrowed equipment for this purpose. Relatively little skill and effort is needed to manually install Typar inserts because they are lightweight and require the same skills used in the installation of common garden and landscape fabrics for weed control. The only difference is that trench inserts are secured vertically in the trench with pins inserted at the top of the wall of the trench, instead of perpendicular to the ground surface as with landscape fabric.

Most rocksaw trenchers used in residential neighborhoods or urban areas cut relatively narrow trenches that may be too narrow to allow easy installation of trench inserts. Rocksaws are the main trencher type used in cities because they are small enough to maneuver within the tight spaces found in neighborhoods and they do not tear up the ground and lawns as badly as the large, heavy chain trenchers or back hoes. One possible solution is to attach small clip-on weights to the bottom edge of the fabric (at intervals along the full length) to help the fabric fall to the bottom of the trench. Also, long lightweight poles such as bamboo, wooden dowels, or half-inch PVC pipes could be used to help push the fabric to the bottom of the trench. Keeping the fabric pulled tightly as it is being installed also helps in lowering it down into the trench. Trenches cut with a backhoe or the larger chain trenchers are much wider than those cut with rocksaws and allow a person to climb down into the trench to straighten and work the fabric to the bottom of the trench.

Another common problem in urban areas is the difficulty of installing trench inserts where there is an abundance of buried utility pipes. This problem involves the question of how to cut the insert material and then get a good seal around utility pipes to prevent roots from growing through these breaks in the fabric. A good approach is to assess whether the pipes are closest to the top or the bottom of the trench. The insert fabric should be cut from the edge of the material that is closest to the pipe to minimize the length of the cut required. The fabric at the cuts should then be overlapped a few inches and stapled, followed by taping with a very sticky wide tape after the insert material is secured with pins into the upper wall of the trench. If the utility pipes are concentrated in a short section of the trench, it may be easiest to just install the inserts in the long continuous sections of the trench that have no utility pipes. There is a low probability that a trench breakout will occur in a very short section of the trench with utility pipes compared with longer sections of the trench.

DISCUSSION AND CONCLUSIONS

Most oak wilt specialists acknowledge that the formation of new root grafts across an oak wilt suppression trench may eventually occur, leading to a breakout some years after a trench is installed. In the author's opinion, this phenomenon is not only common, but increasingly prevalent three or more years after trenching, even in dry sites, especially for live oaks that have such a strong propensity to form root grafts in the shallow soils of central Texas. Oak roots in this region on the Edwards Plateau commonly grow through layers of limestone permeated by pockets of soil. Soil usually fills holes in the rock formed by the percolation of ground water

through the limestone. Nevertheless, the limestone bedrock layer tends to restrict and concentrate oak root growth above it, increasing the chances for root graft formation between the roots. In this situation, trenches alone are not intended to provide long-term protection against root transmission because new root grafts are expected to form over time between these concentrated roots that grow within trench backfill soil.

The current oak wilt breakout rate for TOWSP trenching projects ranges from 21-40% (on a whole-trench basis), depending on location and conditions, suggesting that there is still room for significant improvements in trenching technologies utilized for oak wilt suppression. These rates of trenching failures (breakouts) may not appear very high, but they are very significant for a highly-damaging necrotrophic fungal pathogen that is capable of killing living trees in a relatively short period of time (less than a year) after infection. The ability of *C. fagacearum* inoculum (spores and hyphal fragments) to travel within the transpiration water of oak roots up to 100 feet (30 m) or more per year, beyond the expanding edge of infection centers, further exacerbates the accumulation of damage and mortality to oaks caused by this disease.

Given the current rate of trench breakouts, the oak wilt pathogen has at least one chance in three (during the first two years) of passing beyond any one individual suppression trench installed in Texas using conventional TOWSP methods and trenching recommendations. This rate of disease breakout from trenches is sufficient to maintain the growth of *C. fagacearum* inoculum in oaks and keep the epidemic expanding, especially because numbers of trench breakouts continue to increase over time. For example, there are certain localized areas in Texas where the oak wilt epidemic has expanded to largely unmanageable proportions (Billings et al. 2001). In these areas of very high oak wilt density, the best chance for slowing the epidemic has been for the disease to simply burn itself out as a result of high oak mortality, leaving relatively few susceptible trees to maintain the expansion of the epidemic. Thus, new significant trenching methodologies are sorely needed to further decrease the failure rate of trenches and reduce the spread of individual oak wilt centers. The utilization of new integrated methods besides trenching, such as statewide restrictions on the intercounty transport of firewood from counties with oak wilt and the development of advanced epidemiological models to track movement of the pathogen, also would be useful to focus control implementations to help prevent the spread of oak wilt into other Texas counties and possibly other southern states.

Trench inserts provide a significant new method for improving trenches for oak wilt suppression in the U.S. The benefits of trench inserts could be substantial if they prove to be an effective means for reducing the rate of trench breakouts over long time periods. There are at least two possible strategies for utilizing trench inserts for oak wilt control. The first strategy could be used in situations where the additional costs of applying trench inserts would not pose a significant financial burden due to an abundance of available funds for the trenching project. In this case, trench inserts could be installed in every instance where the available funds for a trenching project are not limited, and the minimal extra expense for inserts could provide greater insurance against future trench breakouts. The second strategy takes into consideration that funds available for the trenching project are limited and the additional costs of trench inserts would pose a significant financial burden. In this case, trench inserts would not be used in the primary trench, but would only be considered if the primary trench failed in the future. Then, trench inserts could be installed in the new backup trench to enclose the breakout and improve long-term security against a secondary breakout. This approach is much more practical and feasible in the majority of oak wilt trenching projects where funding is limited.

Wilson and Lester (2002) reported some experimental evidence demonstrating the efficacy of trench inserts for increasing the effectiveness and longevity of trenches, and providing long-term oak wilt control beyond the first few years after trenching. This article was published at the completion of a 6-year trenching study during which preliminary results of the trench-insert tests were reported (Wilson and Lester 1996a-c, 1997, 1999). The utility of trench inserts has been shown to lie primarily in the prevention of oak wilt root transmission by precluding the formation of new root grafts across the trench within trench backfill soil, two or more years after trenching. The effectiveness of trench inserts in oak wilt suppression programs is being further evaluated by the TOWSP. Trench inserts have been installed in more than 20,000 linear feet (6,153 m) of oak wilt suppression trenches installed by TFS personnel, working within the TOWSP since 2004. Because more than two years are required for roots to grow across trenches and for inserts to show efficacy, the impact of these inserts has yet to be determined. Data from future post-suppression evaluations in the coming years hopefully will provide more conclusive evidence of the performance and effectiveness of trenches containing trench inserts compared with conventional TOWSP trenches without inserts.

The Typar trench insert material is recommended here as the best, most cost-effective material currently available as a water-permeable physical barrier for installation within oak wilt suppression trenches. This lightweight material is relatively easy to install (compared with the other trench-insert materials), comes in a variety of widths, is very cheap (only 40¢ per linear foot), and works as well as Biobarrier based on experimental tests (Wilson and Lester 2002). Typar is a spun fabric that probably performs better than woven fabrics due to the potential for root tips to penetrate holes between the weave in some woven fabrics. Other water-permeable insert materials may have utility as trench inserts, but no other materials besides Typar are currently recommended until they can be properly tested and evaluated both experimentally and in TOWSP field trials.

There are a number of advantages of utilizing water-permeable trench inserts over conventional methods of installing trenches without inserts. Trench inserts eliminate the need for expensive backup trenches, required when primary trenches fail and oak wilt breakouts occur beyond the trench. Trench inserts also provide greater security (insurance) against breakouts in high-hazard sites with large valuable trees, or where symptomatic trees are not removed inside the trench. The additional cost of the Typar water-permeable trench insert is low, on a percentage basis (2-11%), relative to conventional average TOWSP trenching costs in urban, suburban, and rural sites. Typar is available in a variety of thicknesses and prices, providing a range of root-puncture resistance for applications with various oak species, and varying levels of protection against root penetrations. The most expensive trench insert barrier, giving the greatest protection against oak wilt root transmission, is provided by Biobarrier (Typar with trifluralin nodules) which delivers both herbicide and physical barriers to root penetrations.

There is also a strong advantage of having trench inserts installed when trenches must be placed (because of land-access constraints and multiple property lines) far out in front of the infection center; over 150 feet beyond symptomatic trees (not recommended or approved by TOWSP). The longer the time it takes for the trench to be challenged, as inoculum of the fungus approaches the trench in the root system, the more advanced new root grafts will be developed when the fungus finally arrives at the trench. This is the reason why it is not normally recommended to have buffer zones greater than 100 feet from the infection center to conventional trenches without trench inserts.

The ultimate success of integrating trench inserts into the trenching process for oak wilt suppression depends on whether progress can be made in overcoming logistic hurdles such as achieving public awareness of this new disease control strategy, communicating cogent explanations of trench-insert applications and effectiveness, and resolving problems associated with effective implementation (such as trenchers that backfill the trench immediately). First, increasing public awareness and education on trench-insert alternatives and the large potential for improved performance of trenches provided by trench inserts are essential. The inclusion of discussions on trench-insert installations within oak wilt suppression training courses and seminars is needed. Continued expanded field testing of trench inserts within the TOWSP as opportunities arise will facilitate the evaluation of efficacy vs. conventional trenching methods. Also, greater utilization of trench inserts by private oak wilt suppression businesses, and familiarity by trenching contractors will be necessary to streamline the integration of trench insert installation methods into mainstream suppression practices as much as is possible in urban and rural settings.

Ultimately, the development of new trenchers designed to cut trenches and dispense trench insert materials into the trench in one simultaneous operation would be most beneficial. It should be possible to design a trencher system that contains a lightweight, detachable trailer (in tandem) with a vertical post containing a bolt of trench insert material that could be dispensed as the trench is being cut. The trailer could be detached from the trencher when the trencher is serviced or moved to a new location. The advantages of simultaneous installation of trench inserts during the trenching operation include the avoidance of trench cave-in problems and hazards associated with leaving the trench open for extended periods of time.

Trenching as a disease-control strategy continues to be the easiest and most effective option for reducing oak wilt root transmission and tree mortality in localized areas. The direct control of insects or other potential vectors that carry the pathogen, presumably involved in the creation of new oak wilt infection centers, is not readily feasible other than to avoid the wounding of trees when vectors are active. Applying chemical or biological agents to reduce contact between potential vectors and inoculum sources such as fungal mats also is not feasible because of the very large number of red oaks that must be treated. Some currently unknown vectors may have the potential to directly penetrate bark and transmit the pathogen (Wilson, Lester and Edmonson 2000). If this is occurring randomly in forest and urban stands, then there is no feasible means of protecting trees from primary infections. The best opportunities for control in such cases would be to eliminate the sources of *C. fagacearum*-inoculum from which vectors acquire the fungus. However, this may not be feasible if there are too many sources of infected trees in a localized area, but it should be feasible in areas where oak wilt-infected trees are rare in the landscape.

I previously proposed the implementation of state quarantines to prevent the intercounty transport of *C. fagacearum*-infested firewood into counties not affected by the disease (Wilson 1995). This strategy, if implemented, would help eliminate the dispersal of inoculum by human means and reduce the incidence of inoculum transport and vector transmission by natural causes. Although there have been some research efforts to identify oak wilt resistance in live oaks (Greene 1995, McDonald et al. 1998, Gray, this proceedings), none has led to the development of oak wilt resistant lines because no resistance genes have been identified. Host resistance generally is a good long-term disease-control strategy, but in the case of oak wilt, sufficient time does not exist to regenerate resistant or tolerant mature oaks because current rates of oak mortality probably are eliminating mature susceptible trees from the landscape at a rate faster than mature oak wilt-resistant or tolerant oaks could be generated – ranging from 50 to 80 years

(Jacobs 2006). Planting fast-growing native hardwood species immune to oak wilt is a better strategy.

Trenching will likely continue to be the most important and effective oak wilt suppression method used in the foreseeable future as oak wilt incidence continues to increase and become more important in urban areas (Wilson et al. 2004). The continuous development and implementation of new, more effective tools to monitor and control oak wilt are essential for improving our success in managing this devastating disease (Appel and Maggio 1984, Appel et al. 1989, Appel 1995, Wilson and Forse 1997, Wilson and Lester 2002, Wilson, Lester and Oberle 2004, Wilson 2005, Wilson, Lester and Oberle 2005).

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Table 1. Incidence of oak wilt disease breakouts from Texas Oak Wilt Suppression Project (TOWSP) trenches relative to time following trench installation without trench inserts.

Years after trenching	Oak wilt breakouts[1]	Breakout % of total	Cumulative % of total	% change in slope
0.5	17	6.85	6.85	–
1.0	35	14.11	20.96	105.9
1.5	47	18.95	39.91	34.3
2.0	50	20.16	60.07	6.4
2.5	33	13.31	73.38	-34.0
3.0	29	11.69	85.07	-12.1
3.5	17	6.85	91.92	-41.4
4.0	8	3.23	95.15	-52.9
4.5	4	1.61	96.76	-50.0
5.0	6	2.42	99.18	50.0
>5.0	2	0.82	100.00	-66.7
	total 248			

[1]Data are derived from TOWSP post-suppression reports on 248 trenches installed from 1988-1992 when trench depth was 32-36" deep (TOWSP, personal communication).

Table 2. Most probably causes of oak wilt disease breakouts from trenches over time following trench installation.

Trench breakouts occurring within 2 years (60 % of trench breakouts)[1]	**Trench breakouts occurring after 2 years (40% of trench breakouts)**
Improper trench placement (fungus already past; insufficient buffer zone)	Newly-formed root grafts in backfill soil (due to new root growth in trenches)
Insufficient trench depth (fungus passes under trench)	Movement across trench by vectors (above-ground transmission)
Discontinuous trench (fungus passes through gaps in the trench)	Movement across trench by other means (e.g. firewood cut from infected dead trees)

[1]Trench breakout rates are based on trench installations determined from TOWSP post-suppression data and trench-failures indicated by trench breakouts.

Table 3. Experimental incidence of oak wilt disease breakouts from trench barriers over time following trench installation with and without trench inserts.

	New oak wilt disease outbreaks beyond trench barriers					
	Years after trench installation[1]					
Trenching treatment[2]	**1**	**2**	**3**	**4**	**5**	**6**
Trench + Typar	0	0	0	0	0	0
Trench + Biobarrier	0	0	0	0	0	0
Trench + Geo 30 mil	0	0	0	0	0	0
Trench + Geo 20 mil	0	0	**1**	**2**	0	0
Trench only control	0	0	0	**1**	0	0
No trench control	0	**1**	**1**	0	**1**	0

[1]Data are derived with modifications from research publication (A.D. Wilson and D. G. Lester, 2002. Plant Dis. 86:1067-1074). The values above indicate **new** oak wilt disease outbreaks from trenches by year whereas the values in the paper indicate **cumulative** outbreaks by year that charted disease progress over time.

[2]Three replicate trench segments (320-meter mean length) were prepared for each trenching treatment.

Table 4. Oak wilt trenching project results and summary for Lakeway, TX from 1989-2006.

Years installed	Linear feet range	Trench depth	% of trenches	Post-installation result[1]
1989-1999	600-2314	30-36" deep	10.0	holding up to 8 years
		(10 trenches)	50.0	failed within 1-2 years
			40.0	failed after 5-14 years
1997-2006	465-4610	39-48" deep	42.9	holding up to 5 years
		(14 trenches)	21.4	failed within 1-2.5 years
			35.7	failed after 3-9 years

[1]Trenches that failed are defined as those that had at least one oak wilt breakout that occurred somewhere along the total length of the trench within the time periods indicated.

Table 5. Physical characteristics and costs of trench insert materials per linear foot relative to trenching costs.

Trench barrier	Mean costs $/ l.f.[1]	% of trenching costs		Puncture strength lbs	Weight oz/yd^2
		urban	suburban		
Typar 3401	0.40	2.4	11.5	40	4.0
Geomembrane 20 mil	0.91	5.4	26.2	44	14.4
Geomembrane 30 mil	1.20	7.1	34.6	60	21.2
Biobarrier II (weed)	6.90	41.0	198.9	40	13.3
Biobarrier I (root)	8.84	52.5	254.8	40	13.3
Trenching only (suburban)	10.00	59.4	100.0	–	–
Trenching only (urban)	16.85	100.0	485.6	–	–

[1]Costs values per linear foot for trench inserts do not include additional labor costs, and are based on trench insert materials that are 60 inches wide and trenches that are 60 inches deep. Cheaper prices are possible through volume discounts when insert materials are purchased on bulk rolls and when the total linear feet of trench is increased.

Table 6. Availability and physical characteristics of Typar trench insert materials useful for oak wilt suppression.

Product type	Weight	Puncture strength	Fabric width available (inches)[1]				
and grade	oz/yd^2	lbs (N)	36	48	60	75	other widths
Landscape							
Typar 3201	1.9	18	+	+	–	+	–
Typar 3301	3.0	25	+	+	–	+	–
Typar 3341	3.4	34	–	–	–	–	151
Typar 3401	4.0	41	–	+	+	–	–
Heavy duty							
Typar 3501	5.0	56	–	–	–	–	151
Typar 3601	6.0	67	–	–	–	–	151
Typar 3631	6.3	81	–	–	–	–	151
Typar 3801	8.0	93	–	–	–	–	151

[1]Fabric width availability: (+) indicates this width of Typar fabric is available, (-) indicates this width of fabric is not available. Numerical values indicate the fabric widths available beyond 75 inches.

THE TEXAS COOPERATIVE OAK WILT SUPPRESSION PROJECT: LESSONS LEARNED IN THE FIRST TWENTY YEARS

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ABSTRACT

Live oaks (*Quercus virginiana* and *Q. fusiformis*), prized in central Texas for their stately beauty and welcomed shade, are being threatened by a destructive disease – oak wilt, caused by *Ceratocystis fagacearum*. In 1988, the Texas Forest Service (TFS), the USDA Forest Service, Forest Health Protection (USFS/FHP) and others initiated the Texas Cooperative Oak Wilt Suppression Project. For twenty years, this project has been managing the oak wilt problem through unique partnerships and local cooperation. Goals of the Suppression Project have focused on increasing public awareness about oak wilt, identifying and mapping active oak wilt infection centers, and partnering with landowners to contain oak wilt spread. More than 2 million dollars of federal cost shares have been delivered to participating landowners since 1988 as an incentive to treat expanding oak wilt centers. To date, the Suppression Project has installed more than 3.4 million feet (648 miles) of trenches to control 2,466 oak wilt centers. Of these , 2,156 centers (87%) were cost shared with $2.1 million of federal funds. An economic analysis has documented that the $9.2 million of federal, state, city, and private funds invested in the Suppression Project have yielded an average benefit:cost ratio of 6:1 and saved Texas communities an estimated $55 million in tree removal, replanting, and fungicide costs. Achievements in public awareness also have been substantial. An Internet web page devoted to oak wilt management in Texas (www.texasoakwilt.org) has been developed, representing a partnership among various stakeholders. In an on-going effort, specialists with TFS and Texas AgriLife Extension Service have trained various groups of Master Gardeners/Master Naturalists and International Society of Arboriculture-certified arborists on the basics of oak wilt identification and management. These accomplishments and lessons learned in the last 20 years concerning operational management of oak wilt in Texas are summarized.

Key words: *Ceratocystis fagacearum,* disease management, propiconazole

Live oak trees *(Quercus virginiana* and *Q. fusiformis*) comprise a major component of rural and urban landscapes in central Texas and are highly regarded for their beauty, shade, and forage for wildlife. Widespread mortality of live oaks in central Texas has been recognized for many years (Taubenhaus 1934, Dunlap and Harrison 1949) and oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, was officially diagnosed in Dallas in 1961 (Dooling 1961). But it was not until the late 1970s that this widespread mortality of oaks in central Texas was attributed to the oak wilt pathogen (Lewis and Oliveria 1979, Appel 1995). This realization sparked interest in research on this disease in Texas (Appel and Maggio 1984, Appel and Lewis 1985, Appel et al. 1989, Appel and Kurdyla 1992) and provided the impetus for two important cooperative projects initiated in the 1980s: the Texas Oak Wilt Demonstration Project (1982-1987) and the Texas Oak Wilt Suppression Project (1988-present) (Cameron and Billings 1995, Billings et al. 2001).

Conditions in central Texas have changed since the Suppression Project began. An increasing number of large ranches are being subdivided into 5-100 acre "ranchettes" as more people take up residence in this region of the state. Their presence not only increases property values but also increases the incidence and economic impact of oak wilt (see Rooni, this proceedings). Some 25 years of experience combating oak wilt in Texas have given the Texas Forest Service (TFS) a unique perspective on how to effectively manage this destructive pest problem.

DEVELOPMENT OF THE TEXAS OAK WILT SUPPRESSION PROJECT

Project Proposal

During the final year of the Texas Oak Wilt Demonstration Project, the TFS Forest Pest Control Section (now Forest Pest Management) developed an Oak Wilt Suppression Project proposal and submitted it to the USDA Forest Service, Forest Pest Management (now Forest Health Protection (USFS/FHP)) in September 1987 (see Cameron and Billings 1995 for details). The Suppression Project was initiated in June, 1988.

Technical Advisory Board

To provide project guidance and direction, a Technical Advisory Board was formed, consisting of key administrators and specialists with the USFS/FHP, TFS, Texas Agricultural Experiment Station (now Texas AgriLife Research), Texas Agricultural Extension Service (now Texas AgriLife Extension Service), the cities of Austin, Lakeway, Cedar Park and Round Rock, and a private tree care company. This advisory board first met in December 1987 to discuss the Project proposal and implementation process. Since then, membership has increased from 10 to 16 members and the board has met annually to review Project accomplishments and provide long-term direction.

Objectives

The primary goal of the Texas Oak Wilt Suppression Project is to minimize the spread of oak wilt in rural and urban areas of central Texas. Initially, objectives of the Project were to: 1) initiate and accelerate public awareness campaigns to educate urban and rural landowners of the oak wilt threat as well as prevention and suppression alternatives, 2) identify oak wilt centers in selected suppression areas using aerial surveys and contacts with local landowners, 3) assist with implementation of control treatments by providing technical assistance and federal cost-share funds for approved treatments, 4) conduct post-suppression evaluations to record the frequency of re-infections (breakouts) and assist with retreatments if necessary, and 5) develop and refine a computerized record-keeping system for cataloguing and summarizing detection, ground evaluation, and control information (Cameron and Billings 1995).

In recent years, additional objectives have been added. These include an economic analysis of SuppressionProject benefits and costs, conducting systematic aerial detection surveys over the most severely-infected counties, organizing and conducting the first National Oak Wilt Symposium (Appel and Billings 1995), offering field tours to highlight the economic impact of oak wilt and showcase Project accomplishments, initiating a webpage devoted to oak wilt management in Texas, and developing a long-range strategic plan for oak wilt management in Texas. During the last decade, in response to increasing public demands, Suppression Project

efforts have been expanded from three initially-targeted counties (Hood, Travis, Kendall) to more than 40 counties covering most of central Texas.

Organization

Cameron and Billings (1995) described the development and initial organizational structure of the Texas Oak Wilt Suppression Project. The Suppression Project currently is led by a project director at the Texas Forest Service headquarters in College Station, Texas. He is assisted by an administrative coordinator with TFS Forest Pest Management in College Station and a technical coordinator based in Austin. Field personnel gradually have been added to the project to carry out specific project objectives and to address increasing numbers of requests for assistance in key counties.

The seven oak wilt foresters currently involved in the Project devote 40-80% of their time to oak wilt and the remainder to coordinating and implementing other federal and state programs (forest stewardship, urban forestry, fire suppression). Four TFS urban foresters contribute 5 to 10% of their time toward implementing the Suppression Project. TFS secretarial staff members in Lufkin and College Station provide administrative support to field personnel. In addition, the city of Austin has one full-time oak wilt forester and two technicians to implement project objectives within the city limits and the city of Lakeway has employed an oak wilt forester since 2001. Both positions were initiated with support from Suppression Project partnership grants.

Funding

The USFS/FHP, Atlanta, GA provides federal funding (40% since 2006) for this cooperative suppression project while TFS (37%), city partners (3%), and private landowners in central Texas (20%) provide required matching funds. Federal suppression funds allocated annually for this project have ranged from $168,000 in FY 1988 to a high of $595,000 in FY 1995. In recent years, federal funds have leveled off at $400,000 to $500,000 per year. Each federal dollar is matched by cooperating agencies or private landowners. Thus, the total expenditure for this suppression project currently averages $1 MM to $1. 2 MM per year, including the State, City of Austin, and private landowner contributions. In recent years, federal suppression dollars for oak wilt have become increasingly difficult to capture, due to the longevity of the project (federal suppression projects seldom are funded for more than five consecutive years) and to competition for shrinking funds to address oak wilt in other regions and other major forest pests (e.g., southern pine beetle, gypsy moth, Asian longhorned beetle, emerald ash borer, etc.).

Control Tactics

The Texas Oak Wilt Suppression Project has a two-faceted approach to oak wilt management - prevention and direct control. Prevention is promoted through public education on proper timing of pruning and treating wounds on oak trees (Appel, Anderson and Lewis 1986, Camilli, Appel and Watson, this proceedings), elimination of potential fungal inoculum by destroying diseased red oaks, proper handling of firewood, use of propiconazole fungicide, and planting diverse and resistant tree species. Direct control procedures include detection, field evaluation, and control of expanding oak wilt centers (Cameron and Billings 1995). Project foresters work with individual landowners or neighborhood groups to identify the location of oak wilt center boundaries. If the infection center is well defined and considered containable, and the landowner is willing to implement the suggested control treatment, the Project forester conducts a cultural resource survey (Billings et al. 2001) and prepares a written oak wilt suppression plan. The plan,

together with an estimate of costs and a request for cost shares, is submitted for approval to the Project Director. Upon approval, the treatment is installed under supervision of the Project forester. After the treatment is completed, the landowner or neighborhood organization is reimbursed with federal funds for up to 40% of the treatment costs, not to exceed $1000 per single landowner or $5,000 per oak wilt center with multiple landowners.

Currently, the primary cost-shared control procedure involves installation of trenches, at least 4 feet deep, to prevent continual tree-to-tree spread of the fungus through interconnected live oak root systems. A variety of equipment has been used to install trenches, including rotary rock saws, belt trenchers, back hoes, and ripper bars. Rock saws and back hoes are most often used in urban areas. Ripper bars pulled by bulldozers were commonly used in rural areas prior to 1999, but were replaced by rock saws when the depth requirements were increased from 3 to 4 feet. Trenches should completely encircle the center or tie into natural barriers or recently-dug utility trenches. The trench is placed 100 feet in front of symptomatic trees; at least one apparently healthy "buffer" tree should be included between symptomatic trees and the trench. Trenches are refilled with soil immediately after installation. Trench inserts (Wilson, this proceedings) are available, but are not recommended due to the additional expense nor are they cost shared.

Whenever practical, especially in rural areas, it is recommended to up-root and dispose of diseased and apparently healthy trees inside the trenched area. This practice is seldom applied in residential areas, where fungicide injection of trees within the trench is a preferred option. Cost-share funds also can be used for the removal and disposal of symptomatic red oak trees to prevent fungal mat formation and to remove diseased live oaks in urban areas.

Root-flare injections with the fungicide propiconazole prevents many trees from developing severe disease symptoms, but this treatment does not prevent the oak wilt fungus from moving through the untreated root systems and spreading the disease through a stand of live oaks (Appel and Kurdyla 1992). Also, retreatments may be necessary because the effectiveness of the fungicide apparently does not last for more than two years. Therefore, the primary justification for incorporating propiconazole treatments in the Texas Oak Wilt Suppression Project from FY 1990 to 1996 was to provide landowners an incentive to incorporate fungicides in trenching operations designed to stop the spread of the disease. Cost-share funds or donations of free propiconazole (Alamo®) were applied solely to high-value non-symptomatic trees inside cost-shared trenches. Beginning in FY 1997, fungicides were no longer cost-shared by the Project or provided free by the manufacturer.

Oak Wilt Information System

To track Project activities and accomplishments, TFS designed and implemented a computerized record-keeping system (Texas Oak Wilt Information System or TOWIS) in 1988 (Cameron and Billings 1995). This record-keeping system was written in D-Base III for IBM-compatible microcomputers. Project personnel entered data on personal computers at each field station. They could access their records at any time to keep track of landowner names and addresses, treatment status, and detailed treatment information on individual infection centers. Current data were periodically sent from each field station via electronic mail or diskette to the TFS headquarters in College Station where the master records are maintained.

In 2003, TFS staff members created the new database Central Texas Geographic Information System (CTexGIS*)* which has replaced TOWIS. CTexGIS now houses the databases for oak wilt, the Forest Stewardship Program (FSP) and the Forest Land Enhancement Program (FLEP). This database is linked to the geographic information system ArcGIS® 9.2 to provide a seamless

integration of the temporal and spatial data. All staff foresters in central Texas were given training in use of both CTexGIS and ArcGIS® 9.2. Oak wilt data sets for a given forester are "checked out" periodically by the forester, added to or updated and "checked in" to the general database housed on a server in College Station. Through a series of queries or pre-programmed reports, project administrators and field foresters alike have ready access to data summaries for use in periodic reports, post-suppression and personnel performance evaluations, and economic analyses.

Digital Orthophoto Imagery

In FY 1997, implementation of Suppression Project objectives was greatly facilitated by purchase of digital color infra-red imagery (scale 1:40,000) of much of central Texas from EISYS, Austin, Texas. The imagery is provided on compact discs covering individual USGS 7 ½ minute quadrangles. The CDs will operate on microcomputers running Microsoft Windows 3.1, MS Windows 95, or MS Windows NT. With this resource, Project foresters have access to fairly recent (1995/1996) imagery with 1 m resolution. This digital imagery allows them to generate accurate treatment maps and to delineate the spatial distribution and abundance of available hosts in the treatment area. The CIR treatment maps are prepared with Arc-View software to highlight location of infected trees, planned trenches, existing roads and barriers, etc. These maps also are useful during post-suppression revisits to treatment sites as a means to accurately relocate old trenches. This imagery, now more than ten years old, is to be updated at the first available opportunity.

PROJECT IMPLEMENTATION AND ACCOMPLISHMENTS

Public Awareness of Oak Wilt

Suppression Project personnel are continually involved in efforts to make central Texas landowners aware of the oak wilt problem and available methods of diagnosis, control, and prevention. These efforts can be categorized as public presentations on oak wilt, media events, responses to daily telephone calls from concerned property owners, and individual on-site assists (Billings et al. 2001). Recently, a webpage specific to oak wilt in Texas (www.texasoakwilt.org) was initiated. This web page has been developed and is maintained as a partnership among the TFS, USFS/FHP (Region 8), Lady Bird Johnson Wildflower Center, National Biological Information Infrastructure, Houston Advanced Research Center and the International Society of Arboriculture, Texas Chapter (ISAT). This web page is becoming increasingly popular as a source of oak wilt information. For example, in a single month (March 2007), the web site received 79,000 hits and 19,000 page views. With success of the oak wilt webpage, the oak wilt telephone hot line, established in 1990 with support of the Lower Colorado River Authority (Billings et al. 2001), was discontinued in 2005.

To further promote public awareness of oak wilt and the Suppression Project, three illustrated circulars were published and widely distributed to interested landowners and neighborhood groups. These are titled *How to Identify and Manage Oak Wilt in Texas* (Appel, Filer and Cameron 1990, Appel et al. 2005), *Save Our Shade - A Guide to Cost-Sharing for Oak Wilt Control in Texas* (Texas Forest Service 1990), and *Partnerships and Cooperation Combat Oak Wilt in Texas* (Texas Forest Service 1999). A fourth circular entitled *Oak Wilt: A Guide to Identification and Management* (City of Austin 1994) was published by the City of Austin and

distributed by Project personnel. Also, a MS Power Point presentation and a portable photo display describing oak wilt and Project activities have been prepared for public presentations.

Project personnel, in cooperation with Dr. David Appel, Texas A&M University, organized and hosted the 1992 National Oak Wilt Symposium in Austin (Appel and Billings 1995) and the 1996 North American Forest Insect Work Conference in San Antonio (Billings and Nebeker 1996). In 2007, Project personnel assisted the ISAT with organizing and hosting the 2nd National Oak Wilt Symposium. The impact of oak wilt and Project accomplishments were highlighted in Symposium and Conference presentations and field trips.

Identification and Confirmation of Oak Wilt Centers

To date, oak wilt has been confirmed in six counties in west Texas and 55 counties in central Texas. The latter are located primarily along the Interstate-35 corridor from Dallas-Fort Worth to San Antonio (see Rooni, these proceedings). Detection of oak wilt centers by Project personnel is achieved by conducting aerial survey flights over predetermined areas or by responding to landowner inquiries (see Billings et al. 2001).

Control Accomplishments

Since the Suppression Project began in 1988, a total of 2,466 oak wilt centers have been treated with trenches extending for 3.42 million feet (648 miles or 1,037 km). Of these, 2156 centers (87%) involving 3.22 million feet of trench have been cost shared with federal funds; the remainder involved technical assistance from Project staff without cost shares. Based on feet of trench installed with Project cost shares since 1988, the top 10 counties receiving federal assistance to halt oak wilt spread have been Bosque, Gillespie, Travis, Bandera, Kendall, Williamson, Hays, Hood, Bell, and Kerr County (Table 1). Average cost per foot of trench over this 20-year period among these ten counties ranged from $0.50/foot in Bosque County (mostly rural centers) to $4.13/foot in Travis County (mostly suburban and urban centers). Interestingly, the average cost to install trenches has tripled since 1990, increasing from $1.34/foot for the period 1988-1990 up to $4.11/foot in 2007 for all land-use categories combined.

The cost of installing trenches to suppress oak wilt continues to increase (see McKinney and Billings (1995) for initial treatment costs) and varies markedly with land use classification. In FY 1998, for example, trenching costs ranged from an average of $0.60/ft in rural non-residential sites to $10/ft in urban sites. In suburban and rural residential sites, average trench costs were $2.68/ft and $1.11/ft, respectively (Billings et al. 2001). In comparison, the cost of installing trenches to suppress oak wilt in FY 2006 ranged from an average of $1.65/ft in rural non-residential sites to $22.45/ft in urban sites. In suburban and rural residential sites, average trench costs were $3.32/ft and $4.14/ft, respectively. The average cost of trench installation has increased in all land use categories but particularly in urban areas, where trench costs increased by more than $12/foot. The high cost to trench in urban sites reflects the inherent expenses and liability associated with underground utilities and street repairs.

Much of this increased cost was borne by participating urban landowners, since maximum cost shares paid per center were capped at $1,000 for single landowners and $5,000 for four or more landowners and the federal match was reduced to 40% to cover a state-mandated increase from 10.5% to 26% for indirect costs in FY 2005.

Annual accomplishments, based solely on centers treated and amount of trench installed, steadily increased through the first eight years as the Suppression Project grew in personnel and experience (Fig. 1). Since 1995, the annual amount of trenches installed by the Project has

declined to ca. 150,000 feet/yr (46,154 m/yr), due to various factors. These include reduced levels of cost share funds, increasing costs per foot of trench, increased government restrictions (i.e., cultural resources), shifting of Project emphasis to other objectives (post-suppression evaluations, aerial detection surveys, public awareness), and other demands on Project personnel (stewardship, fire suppression, urban forestry). Also, many of the small, accessible, and easily controlled centers have already been treated.

Through September 30, 2007, $2.5 million of federal cost shares had been reimbursed to participating landowners, representing 40% of the total costs of oak wilt treatments. The majority (80%) of these funds have been used for trenching, the primary means of halting the local spread of individual oak wilt centers in live oak stands. Other treatments receiving cost share funds include tree cutting (6%), uprooting trenches within the trenched area (4%), infected tree removal (6%), and fungicide treatments (4%).

Efficacy of Project Trenches

Procedures for conducting the post-suppression evaluation of Project trenches have been described previously (Gehring 1995). This evaluation has become an annual event to document efficacy of trenches, but is now limited primarily to those installed during the previous 3 years. In the fall of 1998, for example, Project personnel revisited oak wilt sites treated with cost-share funds from 1995 through 1997. The occurrence and frequency of breakouts on 571 oak wilt centers were evaluated in relation to feet of trench, month of installation, equipment type, and months since trench installation.

Results reveal that, on average, 76% of all trenches installed from 1994 to 1997 had no breakouts. Of 690 trenches installed between 1991 and 1994, 67% have had no breakouts. Breakouts, when they do occur, are most likely to become visible within 18-30 months after installation. Interestingly, frequency of breakouts did not seem to be related to month of installation or to equipment type. Breakouts were most often attributed to insufficient trench depth (e.g., roots present beneath the trench), rather than to roots reattaching or growing back across the trench. In the winter of 2008, a random sample of 121 trenches out of 356 trenches (34% sample) installed from 2002 – 2005 were revisited. Twenty-six breakouts were observed for a success rate of 79%. This is the first PSE where all trenches visited were at least 48” in depth and the increase in success is attributable to this increased depth (up to 5 feet (1.5 m) with rock saws and 12 feet (3.7 m) with back hoes) and the experience gained over the years in correct trench placement.

Partnerships

The Suppression Project has promoted and benefited from various partnerships. The City of Austin was a major partner in the Project from its initiation in 1988 until 2000, when city budget reductions caused the city to end its participation. In FY 1998, the council converted two temporary oak wilt positions to permanent ones, increasing the oak wilt staff to four persons. In 2006, the city hired an oak wilt forester and resumed participation in the Suppression Project. During the years the City of Austin served as a partner, the Project reimbursed Austin's Parks and Recreation Department (PARD) $25,000-30,000 for their staff's participation in the Project. Also, Austin's neighborhood associations and citizens were reimbursed up to 50% of their suppression costs for approved trenching projects and diseased tree removal. In 2007, the city opted to finance their oak wilt program entirely with city funds.

From 1989 through 2005, the City of Lakeway and Texas Forest Service personnel worked with 170 Lakeway property owners to install over 34,000 feet (10,462 m) of urban trenches. The

city hired its first forester in February 2001, with a partnership grant from the Suppression Project. During the five years that the city was a partner, nine trenches were installed with 95 cooperators totaling almost 18,000 feet (5,538 m). The grants gave Lakeway officials incentive to tackle the oak wilt problem head-on. The city council stepped up to the plate by increasing funding for oak wilt suppression every year despite rapidly rising costs. Feedback on the oak wilt program has been overwhelmingly positive. Many citizens have expressed the belief that they benefit directly from Lakeway's forestry program. After FY 2005, the city declined further partnership grants, opting to continue funding their oak wilt program entirely with city funds.

As described above, partnership grants also have been provided to the Lady Bird Johnson Wildflower Center and the Houston Advanced Research Center primarily to develop the Texas oak wilt web page (www.texasoakwilt.org).

Master Gardener/Master Naturalist/ISA Certified Arborist Training

In recent years, TFS Project foresters in central Texas have become overwhelmed with phone calls and inquiries concerning oak wilt, many of which do not result in cost-shared treatments. In recognition of this fact, a new approach was taken beginning in FY 2005 to increase the availability of volunteers trained in oak wilt detection, prevention, and control. Training sessions were offered to interested Master Gardeners and Master Naturalists as a means to increase public awareness and to serve as an interface between the public and TFS foresters. In FY 2006 and 2007, TFS staff foresters, in cooperation with Dr. David N. Appel, a recognized authority on oak wilt in Texas, continued these training sessions various locations throughout central Texas.

In other training sessions, several dozen certified arborists with the International Society of Arboriculture were trained in oak wilt diagnosis, prevention, and suppression procedures. Again, Dr. Appel, with the Texas AgriLife Extension Service, and various TFS Project foresters served as instructors for classroom and field training sessions for the certified arborist training. Upon successful completion of this intensive 2-day course, each participant is certified as a "Specialist in Oak Wilt."

It is anticipated that these volunteers will assist TFS Project foresters in screening oak wilt-related phone calls and verifying the presence of oak wilt via on-site visits. It is envisioned that such a cooperative partnership with Master Gardeners, Master Naturalists, and ISA Certified Arborists will facilitate the suppression project by reducing the time TFS foresters now spend responding to inquiries from the general public and local property owners.

Economic Analysis of the Project

In FY 1997, an economic analysis of the Texas Oak Wilt Suppresion Project was independently conducted by J. T. Gunter, previously a forest economist from Mississippi State University. Input information consisted of Project accomplishments (specifically trenching, tree removal, and fungicide injection treatments) and Project costs (salaries, benefits, operating expenses, contracts, cost shares, administrative expenses, and indirect costs) for the period FY 1990 through 1996. Rates and extent of spread, host densities, and average tree diameter data were taken from a previous economic analysis of the Project (McKinney and Billings 1995). Project efforts were divided into four different land use categories (urban, suburban, rural residential, and rural non-residential) as previously defined (McKinney and Billings 1995).

Benefits were computed based solely and conservatively on the basis of those dead tree removal and replanting costs avoided when oak wilt spread was halted in individual centers for 5 years by 1) trenching and diseased tree removal or 2) trenching, fungicide injection of trees

within the trench, and diseased tree removal. For each treated center, benefits were defined as the monies saved by a landowner by cooperating with the TFS to suppress the spread of oak wilt. In turn, costs were defined as the actual cost incurred by the Suppression Project to prevent further spread of the disease center, and incorporated Project operating and administrative costs.

No attempt was made to assess or include the value of the trees saved, as was done in an earlier analysis (McKinney and Billings 1995) or additional benefits (protecting real estate values, reducing air conditioning costs, etc.). Assumptions were that, if no trench was installed, oak wilt would continue to spread at 75 feet per year for 5 years, killing 85% of the oaks in the direction of spread (defined for purposes of this analysis as 50% (urban sites) to 75% (rural sites) of the circumference of the oak wilt center).

Based solely on dead tree removal and replanting costs avoided by halting oak wilt spread for 5 years, the average benefit cost ratios were 6, 14, 8 and 4:1 for urban, suburban, rural residential, and rural-non-residential sites, respectively. Based on fungicide injection, dead tree removal, and replanting costs avoided for five years with Project activities, benefit:cost ratios averaged 6, 16, 8 and 4 :1 for the same land use categories. The average benefit cost ratio for both scenarios was 6:1. This suggests that the Texas Oak Wilt Suppression Project is economically efficient. In other words, the $9.2 million of federal, state, local, and private funds invested in oak wilt suppression since 1988 has saved Texas landowners over $55 million in tree removal and replacement costs, exclusive of the many other benefits derived from keeping existing live oaks alive in the central Texas landscape.

LESSONS LEARNED

The Texas Cooperative Oak Wilt Suppression Project is unique among pest suppression projects in that it was initiated by the USFSW/FHP and the TFS in a region where neither agency previously had a strong presence. In the 20 years since the Project began, a professional staff has been established to assist private landowners over an extensive and expanding area in central Texas with education on oak wilt, detection and evaluation of infection centers, and implementation of control treatments with the assistance of cost-share funds. Through the dedicated efforts of numerous cooperating agencies, communities and individual landowners, central Texans are gradually learning to cope with this devastating disease. Furthermore, lessons the TFS and its cooperators have learned about managing oak wilt should benefit other states faced with this disease or other destructive pests affecting multiple ownerships.

Among the lessons the TFS has learned, both about the disease as it expresses itself in central Texas and about its management, are the following. Despite increased suppression, oak wilt may well be having a greater impact now in central Texas than it was having twenty years ago. This is due to the rapid increase in human population, property fragmentation, urban sprawl, increasing property values, and heightened value property owners now place on live oaks (see Rooni, this proceedings). Foresters working with the disease have learned that there is no typical oak wilt center. Each is unique, involves a different set of landowners with different values and resources, and may spread at widely different rates, thus complicating suppression.

Trench depth, placement, and tree removal within the trenched area are keys to successful suppression. Experience has shown that properly-placed trenches dug at least 4-feet (1.2 m) deep are usually effective for halting oak wilt spread, although failure (breakouts) somewhere along the trench is always a possibility and can be expected to occur in a third of the trenches. Most breakouts occur within two years, indicating poor placement or insufficient depth rather

than root grafting across the trench. Seldom do breakouts signify a complete trench failure – just a weak point that can be addressed with a follow-up trench around the breakout area.

Management of oak wilt is equivalent to management of people, since oak wilt is as much a people problem as it is a disease problem. Public education is essential and never ending. New residents, unfamiliar with oak wilt, continually move to the area and their activities often incite the disease (i.e., pruning in the spring, not painting pruning wounds, storing infected red oaks, not diagnosing the disease in early stages, etc.). The loss of prized shade trees to oak wilt often elicits the standard grief steps in affected property owners: shock, denial, guilt, anger, depression, resignation, acceptance, and finally, hope. TFS foresters have learned to help clients through these various stages, offering reforestation with diverse tree species as hope. A holistic stewardship approach to land management has proven most successful.

The Texas Forest Service, in cooperation with the USDA Forest Service and the Texas AgriLife Extension Service, has learned that partnerships are the key to addressing this forest health problem, be it through the oak wilt web page, Master Gardner/Master Naturalist training, or cooperation with arborists and oak wilt venders. Once enlightened about oak wilt, neighborhoods have taken amazing and myriad steps to seek cooperation, fund suppression, and address the problem.

Finally, sufficient and sustained program funding has been critical. Indeed, oak wilt suppression without money is just conversation. With the proper staff, dedication, resources, partnerships, knowledge, and long-term commitment, anything is possible. The Texas Cooperative Oak Wilt Suppression Project is proof of that.

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Table 1: Summary of oak wilt cost-shared trenches by county in central Texas: 1988 – 2007.

County	Centers	Feet	Cost shares	Total costs	Cost/foot
Bandera	197	272,942	$82,260	$211,285	$0.77
Bell	67	102,265	$66,110	$147,309	$1.44
Bexar	22	36,068	$54,431	$165,676	$4.59
Blanco	39	63,432	$45,419	$113,303	$1.79
Bosque	291	368,951	$88,753	$185,313	$0.50
Burnet	34	75,677	$57,592	$154,104	$2.04
Caldwell	1	3,000	$2,500	$5,000	$1.67
Colorado	8	23,835	$14,301	$34,843	$1.46
Comal	12	32,912	$11,539	$20,613	$0.63
Comanche	15	23,625	$14,178	$31,070	$1.32
Coryell	67	81,560	$32,917	$69,034	$0.85
Dallas	1	675	$1,753	$3,505	$5.19
Erath	69	99,015	$23,638	$48,784	$0.49
Falls	3	4,900	$5,400	$12,200	$2.49
Fayette	12	22,310	$18,692	$38,769	$1.74
Gillespie	216	342,929	$132,694	$342,675	$1.00
Guadalupe	1	3,000	$4,500	$9,000	$3.00
Hamilton	42	48,740	$23,720	$48,823	$1.00
Hays	121	217,948	$246,949	$599,255	$2.75
Hill	1	500	$330	$826	$1.65
Hood	96	158,961	$59,609	$130,477	$0.82
Johnson	1	1,000	$675	$1,350	$1.35
Karnes	1	5,300	$2,500	$10,600	$2.00
Kendall	178	257,690	$165,207	$457,391	$1.77
Kerr	67	100,872	$51,749	$155,841	$1.54
Kimble	6	10,675	$7,350	$20,725	$1.94
Lampasas	30	44,785	$27,662	$64,302	$1.44
Lavaca	5	17,130	$7,709	$15,419	$0.90
Llano	1	1,000	$1,165	$2,330	$2.33
McClennan	37	30,095	$31,104	$58,175	$1.93
Mason	3	5,312	$5,236	$10,472	$1.97
Medina	10	15,898	$5,968	$13,782	$0.87
Mills	38	67,509	$21,207	$45,808	$0.68
Palo Pinto	2	2,800	$580	$1,160	$0.41
Parker	26	36,470	$14,755	$30,208	$0.83
Parmer	20	14,131	$19,304	$40,734	$2.88
Somervell	75	90,505	$25,629	$54,908	$0.61
Tarrant	5	5,840	$2,775	$4,450	$0.76
Travis	187	287,320	$455,416	$1,185,942	$4.13

Uvalde	2	6,000	$5,255	$20,075	$3.35
Washington	1	4,678	$2,500	$5,000	$1.07
Williamson	145	234,626	$273,883	$598,888	$2.55
Wise	1	800	$300	$713	$0.89
Total (43 counties)	2156	3,223,681	$2,115,214	$5,170,137	$1.60

Figure 1A

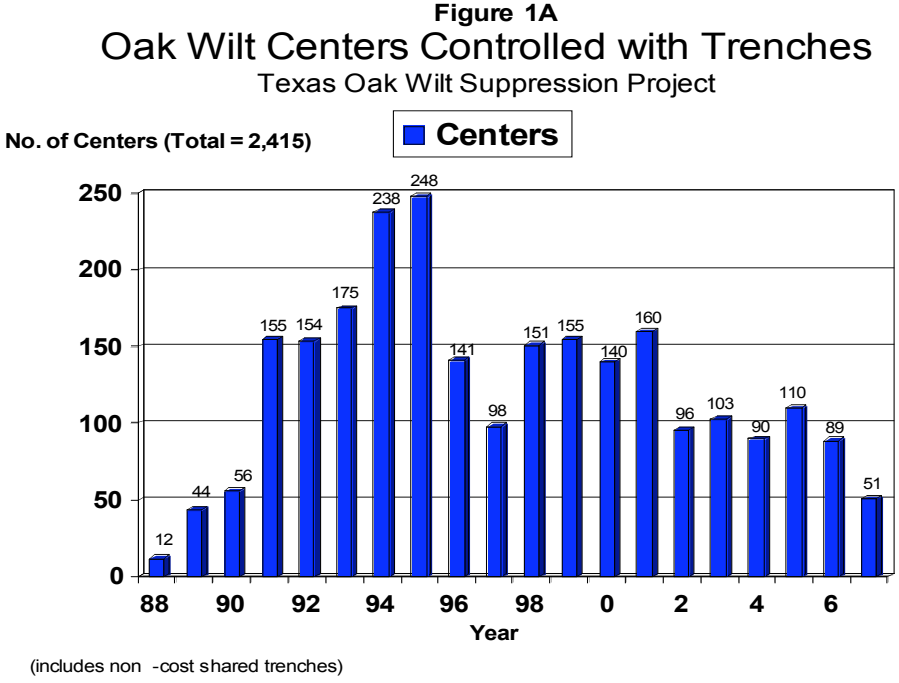

Figure 1B

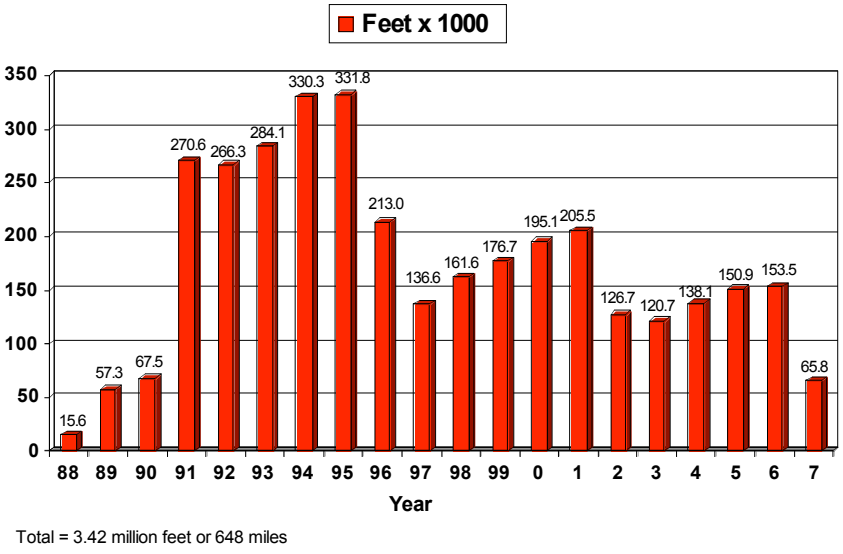

Figure 1: Trenching accomplishments of the Texas Oak Wilt Suppression Project showing oak wilt centers treated (1A) and feet of trench installed (1B) with federal cost shares and/or technical assistance of Project personnel by federal fiscal year.

USDA FOREST SERVICE PERSPECTIVE ON OAK WILT SUPPRESSION

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ABSTRACT

For many years, insect and disease suppression has been a part of the efforts of the USDA Forest Service and its state and federal cooperators in fulfilling our mission to the nation. Various enabling laws have provided authority to cooperatively fund suppression projects. Disease suppression efforts in the U.S. began with the discovery of the introduction of several non-native and virulent tree pathogens which cause such diseases as chestnut blight and white pine blister rust. Both federal and state governments have supported suppression efforts against such diseases. Other diseases have also received attention such as oak wilt and dwarf mistletoes. Cooperative oak wilt suppression programs began in the early 1950s in Pennsylvania, West Virginia, and other eastern states; but by the 1970s they were deemed largely ineffective and unnecessary. More recently, outbreaks of oak wilt in central Texas and southeastern Minnesota have precipitated suppression projects that have had better success and continue at the present time. Funding of cooperative pest suppression projects is provided where a pest presents a significant threat to a major forest resource and the likelihood of success is reasonably high. Availability of funds, competition with other significant pest threats, and politics can often influence funding availability and decisions. Oak wilt suppression projects, like all projects, are considered within this context. While suppression projects remain a fundamental component of the overall USDA Forest Service mission (and that of state agencies, too), prevention activities and early detection/rapid response efforts are being increasingly employed in an effort to minimize the introduction, spread, and effects of insect and disease pests at an early date, before major epidemics can occur.

Key words: *Ceratocystis fagaceaum*, disease management

For many years insect and disease suppression has been a part of the efforts of the USDA Forest Service and its state and federal cooperators in fulfilling our mission to the nation. Various acts of legislation have authorized funding of suppression projects over the years. Our current authority resides primarily in the **"**Cooperative Forestry Assistance Act of 1978, As Amended Through 2002**"** (USDA Forest Service 2005). The Forest Health Protection Section (Section 8, 16 U.S.C. 2104) authorizes many activities related to forest health including suppression. Suppression funding is applied directly on federal lands of all types, but on state and private lands, project funding is cooperative, with states or other entities providing about 50 percent of the funds as a "match" to federal funds.

Matching expenditures can be direct cash outlays or indirect costs such as salary supporting an employee's time, institutional overhead charges, or labor and equipment used in lieu of contracted work, etc. However, matching cannot be made using funds from other federal grants. Most suppression funding is provided to state agencies, although occasionally non-profit, non-governmental organizations are also funded. Historically, insect suppression projects have probably dominated in size and financial scope, but disease projects have been funded as well. And most recently, non-native invasive plant suppression projects have been added to the spectrum.

EARLY DISEASE SUPPRESSION

Forest disease suppression efforts in the U.S. began with the introduction and discovery of several non-native, virulent tree pathogens which caused serious diseases and threatened major forest resources. The first of these was the chestnut blight (Beattie and Diller 1954, Hepting 1976). The fungal pathogen that causes the blight, *Cryphonectria parasitica,* (Murrill) Barr, was first discovered in the New York area in 1904, although it was probably introduced prior to that. It is now known to be of Asian origin. As the blight spread into the native chestnut population in eastern forests, the first suppression efforts came via a state program, not a federal one. The state of Pennsylvania created the Chestnut Blight Commission in 1911 and over a 4-year period allocated over $500,000 to the work. The federal government did play a role, though, and provided funds for research on the disease - $5,000 dollars in 1911 and $80,000 in 1912 and 1913.

The suppression effort in Pennsylvania was attended by much controversy over the potential for success. The skepticism turned out to be well-founded as the blight spread too fast for operational activities to keep up and by 1914, suppression efforts were abandoned. The blight spread mostly unabated and by about 1940 was found throughout the host range of American chestnut. Early interest in disease resistance to the blight was generated by the observation that Japanese and Chinese chestnuts were resistant. Experimental plantings of oriental trees and crosses with American chestnuts began a long-term effort to develop and deploy a resistant replacement to the native tree. This work continues today and test plantings of resistant trees from the American Chestnut Foundation are currently being made on some national forest sites.

White pine blister rust was the second introduced disease to threaten a major North American forest resource (Pack 1934, Hirt 1956, Maloy 1997, Kinloch 2003). This disease is caused by a fungus, *Cronartium ribicola* Fish. and is also of Asian origin. It was introduced first to Europe and then the New York area. The fungus requires an alternate host, *Ribes* spp. (currents, gooseberries), and it was on these that it was first found in 1906. It was later found on planted white pine in 1909 and on natural white pine in 1915. Coming so closely on the heels of the chestnut blight, concern was immediate and control efforts quickly considered. The earliest control efforts, about 1910, were aimed at nursery production in an effort to keep diseased seedlings from being widely outplanted. To make matters worse, the disease was also found introduced to Vancouver, Canada in 1921, adding a threat to the western 5-needle pines.

The threat from this disease was the genesis of one of our earliest forest disease legislative efforts in 1912, the "Plant Quarantine Act". Under this act, Quarantine #1 prohibited the importation of 5-needle pines to the U.S. The Act also enabled states to regulate the movement and cultivation of certain plants—this became the basis for the *Ribes* eradication efforts which were the focus of white pine blister rust suppression efforts for many years. The theory was that eliminating *Ribes* bushes in and around white pine stands would break up the complicated life cycle of this fungus and reduce or eliminate infection. Federal funds were initially provided in the amount of $20,000 in 1916, matching a $21,974 multi-state allocation. *Ribes* eradication grew into probably the biggest, most expensive forest disease suppression effort ever.

Efforts in one or more areas of the U.S. were active for about 50 years, ending in the 1960s with an estimated total expenditure of about $150 million. During the depression years, and the years after, the Civilian Conservation Corps was used as well as groups of men from other work relief programs. An estimated 20 million acres (8 million ha) were treated for white pine blister rust amelioration, a truly stunning amount. Unfortunately, the effect of all this effort was

considered only minimally beneficial in the East and mostly unsuccessful in the West where the disease was more severe. As with the chestnut blight, difficulty in controlling blister rust engendered an interest in disease resistance, especially in the western white pines and breeding and research continues today. This disease continues to be a threat to valuable forest resources, especially in the western U.S.

OAK WILT SUPPRESSION

Other diseases such as oak wilt, the subject of this symposium, have also received attention. Oak wilt, caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt, was first recognized as a threat in the 1940s and 1950s (MacDonald 1995). Early suppression efforts began in the 1950s with programs in Pennsylvania, West Virginia, Kentucky, North Carolina, and Tennessee. Most of these received some federal funding although documentation is scant or difficult to locate. West Virginia and Pennsylvania apparently had the biggest, most active programs. For example, in 1957 West Virginia received about $30,000 as a 33.3% share of a $90,000 project. Federal funding continued for at least these two states for nearly 20 years until the suppression efforts were discontinued, being deemed either ineffective, uneconomical, or both. These programs were summarized at the 1st National Oak Wilt Symposium in 1992 (Haynes 1995, Merrill 1995). U.S. Forest Service research and monitoring of suppression methods was very active at this time and various state programs were intensively studied for effectiveness (Jones 1965, Jones 1971).

Renewed interest in oak wilt suppression surfaced in the 1980s when the disease became widely diagnosed in central Texas live oaks and research efforts began to test and demonstrate effective control tactics. A 5-year cooperative federal-state demonstration project during 1982-1987 in central Texas showed the extent of oak wilt distribution and the likelihood of a successful suppression project (Cameron and Billings 1995). A cooperative federal-state suppression project was initially funded in 1988 with $168,600 federal and matched by state and local expenditures. Since then, the project has been continuously operated by the Texas Forest Service with federal funding increasing to about $500,000 per year (Fig. 1) and is summarized elsewhere in this symposium (Billings, this proceedings). A similar project was also initiated in southeastern Minnesota in 1990 which ran for about 7 years (Fig. 2). After a period without federal funding, cooperative funding resumed in 2002, and continues to the present. These two projects have experienced success in controlling oak wilt due mostly to the uniformity of the host type being damaged. Spread in both areas is primarily by root contacts or grafts and trenching or plowing to sever these grafts does a good job of stopping infection center expansion.

Another disease problem which has received a good bit of suppression funding over quite a number of years is dwarf mistletoe (*Arceuthobium* spp). Most projects have been in the western regions and data on expenditures and locations are scattered and difficult to summarize. But, as an example, one summary of work in the Pacific Northwest documents suppression activities beginning about 1959 and peaking in the 1970s with expenditures of about $400,000 in one year (Hadfield and Russell 1978). Mistletoe control programs remain active and are still being funded.

This year, over $49 million has been allocated for forest pest suppression efforts. Pests include gypsy moth, southern pine beetle, dwarf mistletoes, emerald ash borer, hemlock woolly adelgid, oak wilt, and others. About $600,000 of this has been dedicated to cooperative oak wilt projects. Some of these are listed in Table 1. Other oak wilt projects which are receiving funds (although from a different source of federal funds) are listed in Table 2.

SUPPRESSION PERSPECTIVES

Federal funding of forest pest suppression projects is driven by a number of issues. But, to generalize, projects which successfully receive federal funds usually address a significant threat to a major economic or ecological resource and have a reasonable potential for biological and operational success. Project selection is also affected by (1) the amount of funding available in a given year, (2) the differing pest threats that loom in a given year, and (3) the ever-present wild card of politics (as then State Forester Bruce Miles said in his welcoming address to the 1st National Oak Wilt Symposium, sometimes a project gets funding when a senator or congressman "…explains it better…"; Miles 2005).

When funding levels are adequate, decision-making on federal funding requests by Forest Health Protection is relatively uncomplicated. Our specialists verify the need and potential success of proposed projects and, as long as sufficient funds are available, most projects are approved. When budgets are tight or when huge, expensive suppression needs arise, some projects must be left un-funded and others must do with less-than-requested amounts. Occasionally, federal funding exigencies, such as a disastrous wildfire season, have diverted suppression dollars away from legitimate, worthwhile pest suppression projects.

NON-SUPPRESSION EFFORTS

As a counterpoint to suppression, the U.S. Forest Service also is active in funding, operating, and supporting a number of prevention and early detection programs aimed at minimizing the introduction, spread, and detrimental effects of insect and disease pests at an early date, before major epidemics develop. Some examples of these are the (1) Southern Pine Beetle Prevention and Restoration Program, (2) the Sudden Oak Death Survey Program, and (3) the Early Detection/Rapid Response Program for exotic bark beetles.

The Southern Pine Beetle (*Dendroctonus frontalis* Zimm.) Prevention Program has been funded since 2003 as a cooperative effort with southern states. Nearly $60 million has been allocated to date and all 13 southern states as well as 12 national forests currently have active programs. Efforts are aimed at thinning pine stands early in their life cycle, including pre-commercially, to reduce the hazard to southern pine beetle. Many states are using cost-share incentives to encourage landowner participation. Hundreds of thousands of acres have been treated so far.

The sudden oak death surveys have been a response to the potential introduction of this disease-causing organism (*Phytophthora ramorum* S. Werres, A.W.A.M. de Cock and W.A. Man in't Veld) to other states from California, Oregon and Washington on infected nursery stock (Todd undated, USDA Forest Service 2004). The disease was discovered in California in 1995 killing oaks (*Quercus* spp.) and tanoaks (*Lithocarpus densiflorus*) in coastal and central counties. As the disease problem grew there, it was discovered in 2003 and 2004 that the causal agent was also infecting a large number of nursery plant species in commercial container nurseries and that these potentially-infected plants had been shipped unawares to 49 of the 50 states. Many of these plants were sold before the USDA and state agricultural agencies could get the nurseries inspected and destroy infected plants. The potential for the organism to escape into the natural environment outside of California was instantly huge. The Forest Service in conjunction with state cooperators quickly implemented a large-scale detection survey program looking at the perimeters of nurseries that received infected or potentially-infected nursery stock. Nearby forested areas with potential hosts or forest areas with suitable hosts and climate were also surveyed.

Detection survey work began with 7 states in 2003 and has increased to 38 states in 2006 (Oak et al. 2008). Funding levels have been between $300,000 and $1.3 million annually. To date, no introductions of the pathogen to new wildland areas have been discovered. Since distribution of infected nursery stock has been substantially reduced, the survey efforts are now being reduced in size, scope, and funding although this reduced effort will continue for some time. Since survey results have been negative for 4 years, an alternative detection technique is being used to sample larger areas with less effort. Stream baiting is being used in 2007 to detect the presence of the pathogen in waterways downstream of nurseries or forest areas worthy of survey.

The early detection and rapid response program for exotic bark beetles began in about 2001 with $30,000 in funding and has grown to a $750,000 program in 2007. Bark beetle trapping is currently being done in 17 states in about 120 locations. Traps are placed in proximity to ports of entry, shipping, storage or manufacturing facilities that represent pathways for the introduction of exotic beetles in wood products or shipping materials. A number of exotic beetles have been trapped and identified. One of particular interest was trapped in 2002 at Port Wentworth near Savannah, Georgia (Mayfield and Thomas 2006, Johnson et al. 2007). It was identified as an ambrosia beetle of Asian origin, *Xyleborus glabratus* Eichoff.

Unfortunately, in spite of this "early detection" the beetle has established itself in local populations of red bay (*Persea borbonia*) and sassafrass (*Sassafras albidum*) trees. This beetle, as with other ambrosia beetles, carries a fungus which colonizes the attacked trees and provides food for the beetles. The one carried here is a pathogenic fungus of the genus *Raffaelea* which acts as a vascular wilt, quickly killing infected trees. The pair of pests has rapidly expanded their range into 31 counties in Georgia, South Carolina, and Florida. The host range has also expanded with attacks and infections now known in pondberry (*Lindera melissafolium*), pondspice (*Litsea aestivalis*), and avocado (*Persea americana*) (Hanula et al. 2008).

CONCLUSIONS

In the future, suppression funding and projects will still be needed and will continue to play a significant role in the increasingly complex arena of forest health management and oak wilt projects will probably remain among those funded. However, prevention and aggressive detection programs may play an increasingly important role in a world of fast-paced, global commerce.

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Table 1. Fiscal year 2007 funds (USDA Forest Service) allocated to cooperative oak wilt suppression projects.

Cooperator	Allocation
Texas *($300,000 + $200,000 Southern Region funds)*	$500,000
Minnesota	$200,000
Michigan	$50,000
Wisconsin	$50,000
Chequamegon-Nicolet NF	$25,000

Table 2. Fiscal year 2007 funds (USDA Forest Service) allocated to other federal installations for oak wilt suppression projects.

Federal Installation	Allocation
Fort Hood, Texas	$70,000
Balcones National Wildlife Refuge, TX	$24,000
Army Corps Engineers, St. Paul District	$1,330
Fort McCoy, WI	$40,000

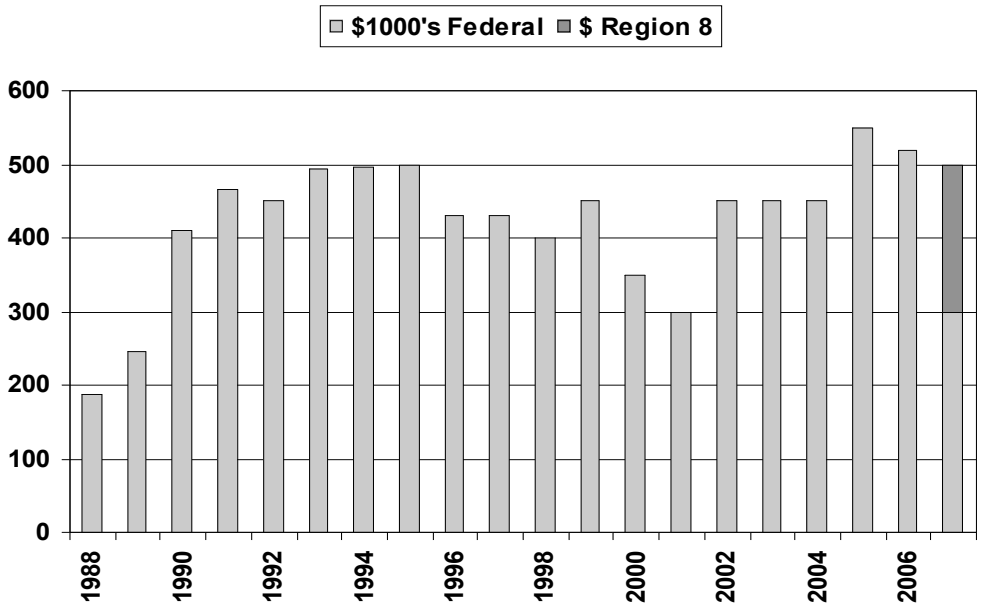

Figure 1. Federal (USDA Forest Service) dollars allocated to the Texas Cooperative Oak Wilt Suppression Project.

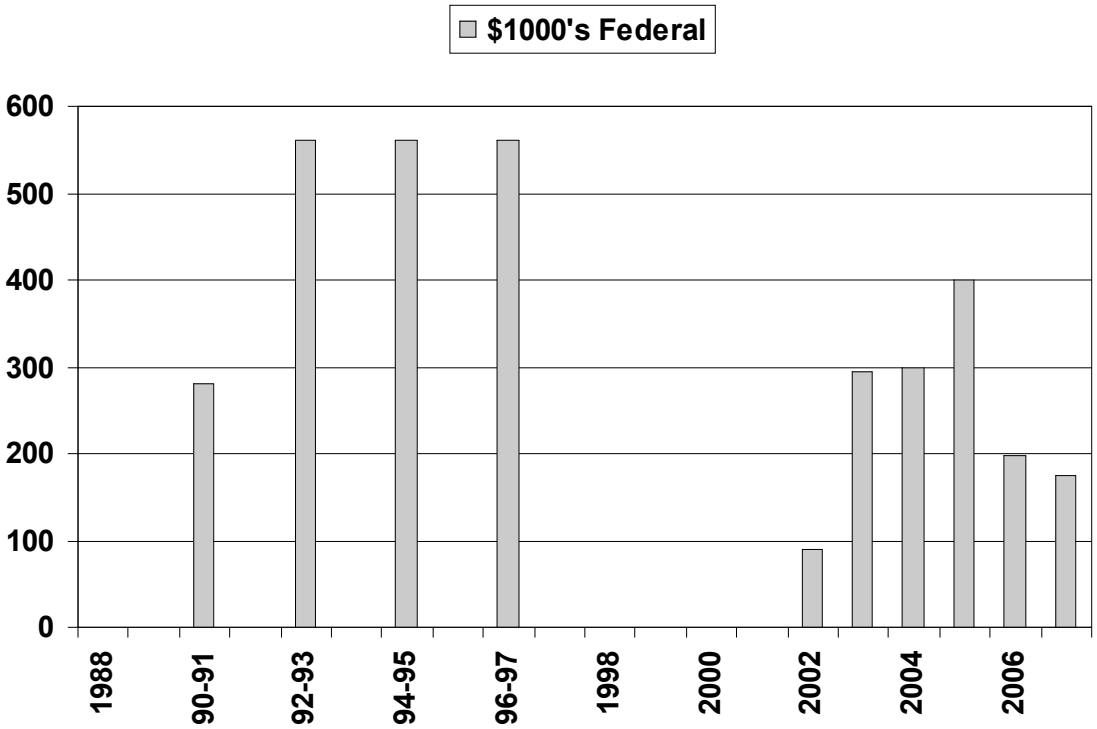

Figure 2. Federal (USDA Forest Service) dollars allocated to the Minnesota Cooperative Oak Wilt Suppression Project.

SECTION IV

OTHER THREATS TO OAKS

RECENT FINDINGS AND CURRENT PROSPECTS ON THE BIOLOGY AND MANAGEMENT OF *PHYTOPHTHORA RAMORUM*

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ABSTRACT

Exotic *Phytophthora* species represent a significant threat to native North American oaks. The diseases they cause may range from extremely virulent aggressive pathologies to secondary diseases in need of further weakening factors in order for plant mortality to occur. The severity of the disease may depend both on the pathogen and host species. Exotic Phytopthoras can further be divided in two groups; one of recently discovered introduction and one of species long known in the agricultural world, but only recently-discovered in wild environments in some North American regions. Population genetics information can be deployed to determine linkages between agriculture and wildlands, and at times surprising results about the role played by specific strains may be obtained, indicating that particular emphasis may be needed to prevent introductions of pathogen genotypes linked to some specific source.

Key words: oak disease, population genetics, sudden oak death

Phytophthora ramorum is the exotic pathogen responsible both for sudden oak death (SOD) in California and Oregon coastal forests (Rizzo and Garbelotto 2003), and for a *Phytophthora* blight disease of a wide array of ornamental plant species in commercial nurseries throughout North America and Europe. There are at least three review papers on this topic (Garbelotto and Rizzo 2005, Rizzo, Garbelotto and Hansen 2005), and I invite the reader to consult those for bibliographical references. Nonetheless, I will include those references that are not listed in any of the three review papers. Because of its high virulence, *P. ramorum* is highly regulated, and has been studied intensively since its role as the causal agent of SOD has been uncovered.

The origins of the organism are still unknown, but its genome sequence (Tyler et al. 2006) has revealed that the European and North American strains are characterized by heterozygosity levels typical of individuals originating from a sexually reproducing population. The whereabouts of these sexual native *P. ramorum* populations are still unknown. At least three studies employing a range of genetic markers, such as AFLPs and microsatellites, have clearly indicated though that sexual reproduction is not ongoing in North America and is not likely to be occurring in Europe. The high levels of heterozygosity evidentiated by studies employing microsatellites suggest that the current known lineages of *P. ramorum* are the results of mating between genetically-divergent genotypes followed by prevalent clonal reproduction.

Two lineages of the pathogen were at first described: one present across European nurseries and one in California forests. These two lineages are genetically and phenotypically distinguishable, as indicated by their different allelic composition and mitochondrial DNA sequence, colony morphology, growth rate, and mating type (North American isolates belong to mating type A2 and with two exceptions all European isolates belong to mating type A1). While these differences indicate a lack of a direct connection between the California wildland infection and the European nursery infection, both lineages are reported to be present in some North

American nurseries. A further third lineage, genotypically and phenotypically distinct from the other two, is also present exclusively in some North American nurseries.

The microsatellite study by Ivors, Garbelotto et al. (Ivors et al. 2006) also identified individual genotypes within lineages: while the California infestation appeared to be caused almost exclusively by one genotype, multiple genotypes were identified in European and North American nurseries. This pattern may be explained by multiple introduction events of individuals belonging to distinct lineages into the commercial nursery trade in both continents, while the introduction into the wild can probably be associated with a single or multiple introduction of a few very closely-related genotypes. The fact that the California wild genotype is one of the several present in U.S. nurseries, and the fact that U.S. nurseries contain all three known lineages of *P. ramorum* suggest that nurseries may have operated as a stepping stone for the introduction of the pathogen into the wild.

We have furthered the genetic analysis of *P. ramorum* lineages and populations by using two distinct approaches. First, we have sequenced the flanking regions of five microsatellite loci and performed an analysis of the combined dataset for three genotypes, each belonging to one of the three lineages. Second, we have used three newly developed hypervariable tetrarepeat microsatellite loci to study hundreds of individuals from several populations scattered throughout the entire zone of infection in California, and of isolates found in nurseries across North America. In this analysis, we also included two sets of isolates that were obtained in 2002-2003 from several locations in two California counties (Santa Cruz and Sonoma), where SOD was reported since the late 1990s.

The combined loci sequence analysis revealed that each one of the six haplotypes (two haplotypes for each diploid genotype) fell in one of two clades. One clade included one haplotype from the European lineage, one haplotype from the North American lineage, and both haplotypes of the third lineage. The other clade included one haplotype from the European lineage and one from the North American lineage. This analysis helps to better understand the history of the known lineages of *P. ramorum*, indicating that both the North American and European lineages are the result of mating events between different genotypes belonging to one of the two clades. On the other hand, the third lineage appears to be the result of mating between two haplotypes belonging to the same clade. The third lineage in fact is the only one to be characterized by some alleles displaying lower than expected heterozygosity levels.

By using the more sensitive tetra-repeat microsatellite markers, we have been able to increase our ability to differentiate genotypes. From the two originally identified, the number grew to over 40. It should be highlighted that these 40 genotypes are almost identical or quasi-clonal, and in the absence of sex they must have originated through mutation or somatic recombination events. The ability of an organism to generate new genotypes in the absence of sex will be directly correlated to a) the mutation or somatic recombination rate characteristic for that species, and to b) the size of its population. Because mutation rates are normally constant for a given species, the number of new genotypes is likely to be correlated to the actual size of the population. *P. ramorum* has been in an unchecked epidemic level for the last ten years, and its population in the wild has likely reached an enormous size (millions of trees have been estimated to have been infected yearly by the pathogen). Therefore, it is not surprising that the number of genotypes in the wild is currently larger than that in nurseries, where the disease is intensively managed and controlled.

A contingency analysis based on the frequency of genotypes detected in each of the study sites indicated that, with one exception (see below), the frequency of genotypes at each study site

was significantly different. This was also true for sites only 20 km apart, suggesting that the number of migrants between these sites is not sufficient to homogenize genotype frequency. Furthermore, over 50% of the genotypes were "private", i.e., exclusively found in individual sites, indicating a great extent of population subdivision in the zone of infection in California. Although *P. ramorum* disperses aerially, the population structure here described indicates that long distance aerial movement is a rare event. This pattern is distinctively differently from that described for other aerially-borne oomycetes. While the frequency of genotypes is distinctly different among sites, and many private genotypes were detected, some genotypes were considerably more common than others: those are likely to be the genotypes originally introduced.

The presence of identical genotypes in multiple locations suggests that some site to site movement is occurring, but that this movement may be rare or still unidirectional because the introduced pathogen has not yet reached its equilibrium in its new range. One striking exception was found when comparing the genotypic frequency among all locations: two sites, approximately 100 km apart, one North (Mount Tamalpais. Marin County) and one South (Scotts Valley, Santa Cruz County) of the Golden Gate were found to have an undistinguishable genotype frequency. There is corroborating evidence that infected ornamental plants were sold from the Santa Cruz to the Marin County area. Movement of infected plants between the two areas may explain the similarity in pattern between these two distant sites.

When we compared the frequency of genotypes found in nurseries, with those found in 2002-2003 in Santa Cruz and Sonoma Counties, we found no differences between genotype frequency of wild and nursery populations; this finding supports a close link between nursery populations and the early SOD epidemic. In light of the large number of *P. ramorum* individuals in the current pandemic in California, the contribution of possible current escapes from the nursery in the wild is probably insignificant. However, just recently a genotype associated with nursery plants was detected in a California stream outside the zone of infection, indicating that past introductions associated with nurseries may have not all been detected yet, or may be currently occurring. Some of the genotypes detected in the wild in 2002-2003 were not detected again after 2004; on the other hand novel genotypes are at times found. Because of potential extinction events and the creation of new genotypes through mutations, *P. ramorum* populations appear to be shifting as time goes by.

While long distance aerial movement is probably an infrequent, but not rare event, short-distance aerial movement is responsible for the frequent local spread of the pathogen. Although the extent of local movement has not been adequately studied, it appears to range between 10 m, in the absence of wind, to approximately 3,000 m. Several studies have indicated that sporulation of the pathogen mostly occurs during the rainy season, and that inoculum load is directly correlated with number of California bay laurel (*Umbellularia californica*) present in a site. The above information is based on collection of sporangia from rainwater under bay laurel and under other plant hosts, and on the observation of sporangia from symptomatic plant tissue collected in the wild. We have monitored inoculum levels for 2 years in 8 study plots using a different approach. A total of 16 buckets were placed in each site, and infection was monitored by baiting 5 bay leaves in each site. In the lab, we had previously determined that infection of up to 3 leaves corresponded to low and medium levels of inoculum, while infection of 4 or 5 leaves corresponded to high levels of inoculum. Leaves were left in the buckets for 3 weeks, and water was placed in the bucket, so that measurements would be independent of rainfall. Results indicated that low level of infection is possible in the absence of rain, but infection levels peaked

during the rainy season, but almost exclusively in the warm March to June months. Almost no infection was detected in the colder months of January and February in spite of the fact that rain events occurred in that period, indicating that while sporangial production may occur in colder weather, infection is favored by warmer temperatures. Infection levels were also much higher where bay density was higher. It should be highlighted that the sites with lower bay densities were characterized by the presence of tanoaks (*Lithocarpus densiflorus*). This indicates that tanoaks are not equivalents to bay laurels in sporulation ability.

Questions such as how long the pathogen sporulates on individual hosts and how long the pathogen may survive during the unfavorable dry summer and fall seasons are questions that have not fully been answered yet. The survival issue is one that may vary significantly year to year, and may be highly dependent on the type of substrate (soil vs. leaves vs. wood vs. water). One complexity of addressing the survival issue is that of differentiating samples in which the pathogen is dead from those in which the pathogen is dormant. Neither culturing nor DNA-based assays can differentiate between these two. We have developed a reverse transcriptase (RT-PCR) assay that targets mRNA of the COX mitochondrial region. COX mRNA is highly specific to *P. ramorum*, and is an indicator that the organism is breathing. RT-PCR results indicated that culturing significantly underestimates survival, while DNA-based assays overestimate it. By using this technique, we have determined that in 2005 an average of 40% of bay infections were still active in October, but this percentage varied greatly among sites. It should be noted that 2005 was a particularly favorable year for *P. ramorum,* and that survival percentages may be a lot lower in drier years.

One of the complexities of SOD is represented by its life cycle: while oaks (*Quercus* spp.) and tanoaks are lethally affected by the disease, they are not the most infectious hosts. California bay laurel, a species that only develops a foliar blight when infected, is the so-called SOD "superspreader." The fact that the disease does not seem to significantly reduce the fitness of bay laurels is unfortunate, because when environmental conditions are favorable, epidemics can develop basically unchecked. Some ornamental host such as camellias (*Camellia* spp.) and rhododendrons (*Rhododendron* spp.) are also known to be able to spread the disease, and the few wildland infestations in Europe appear to be mostly associated with the presence of the latter genus. While the role of bay laurels in the epidemiology of SOD is supported by various lines of evidence, *P. ramorum* can infect an extremely broad range of hosts: the role played by most of these hosts in the disease epidemiology is still unknown. In order to determine the relative importance of each host in the wild as a substrate of colonization by *P. ramorum*, we have collected approximately 400 samples from 41 symptomatic plant species in 7 sites in California. Approximately 300 samples from 30 species were confirmed as infected by *P. ramorum* both by culturing and DNA-based assays.

The most important host for the pathogen was California bay laurel (57% of all confirmed infections), followed by tanoak (14%), redwood (*Sequoia sempervirens*) (5%), hazel (*Corylus* spp.) (4%), toyon (*Heteromeles arbutifolia*) (3.5%) and Pacific madrone (*Arbutus menziesii*) (3%). Although the sporulation potential by *P. ramorum* has not been determined for all of these hosts, it is likely that at least redwood, hazel and Pacific madrone may play a role in the spread of the disease. It is plausible that these hosts may be important in the *endemic* spread of the disease across the landscape, by allowing the pathogen to become established in new sites. These hosts in fact appear to be commonly infected and do not require the presence of bay laurels for infection to occur. Other hosts, such as Douglas-fir (*Pseudotsuga menziesii*), or many of the herbaceous plants and ferns normally appear to be infected only when infected bay laurels are in

the same area. These hosts may fundamentally "bait" the pathogen only when inoculum conditions are very high (epidemic levels driven by the presence of infected bay laurels), and may not play an important role in the spread of the disease in nature.

Controlling SOD in the wild has not been an easy task. Even after relatively dry and unfavorable weather for the pathogen, rapid climbs in population levels have been noticed following just a couple of repeated rain events. On the coast of California, the percentage of infections surviving from one season into the next may be rather high. Two control approaches have been pursued: in Oregon, where infection was discovered in its early stage, a continuing effort to drastically reduce inoculum is attempted on a yearly basis. This effort includes cutting of all infected plant species, herbicide and fungicide treatments, and burning of infected material. Although the disease has not been eradicated, inoculum levels have remained consistently low.

In California, where the disease already occupied hundreds of miles when the causal agent was identified, mitigation efforts include protective chemical treatments of oaks and tanoak, reducing the number of bay laurels, and sanitation efforts using prescribed burns, chipping infected woody material, and composting. Phosphites have been mostly applied as bark sprays in conjunction with the organosilicate surfactant Pentrabark: Recent data have indicated that the effects of a single treatment last at least 20 months. Furthermore, when treating tanoaks, a reduction in foliar lesion development (those lesions are responsible for the sporulation of the pathogen), has been documented. Whether resistant oaks and tanoaks exist is still an open question, but the observation that oaks are infected only in the proximity of infected bay laurels and the reported presence of less susceptible individuals in each of nine populations studied, may indicate that oak species are not at risk of going extinct. On the other hand, although variation in susceptibility has also been recorded for tanoaks, it has been shown that some populations are on the whole more susceptible than others.

The high levels of mortality, locally approaching 100% of adult individuals, suggests that these susceptible populations are at risk of disappearing. A large common garden experiment has been started with the aim of identifying potential resistance traits (patterns of susceptibility indicate that infection of tanoaks and oaks by *P. ramorum* is a multi locus trait) that may be used to rear seedlings for restoration projects. In terms of sanitation, it has been shown that chipping and drying infected woody substrates is effective, and also that composting is effective, but more research needs to properly address the effects of fire. Finally, the impact of thinning California bay laurel is unclear, and studies are under way to address this issue.

Although a valiant effort to stop the disease is under way in the known areas of infection, the best strategy remains preventing its introduction in new areas. The reports in 2007 that *P. ramorum* was baited from two rivers in two states outside of California and Oregon is troubling. International and national agencies have placed a high priority in preventing further spread of the pathogen, and a series of surveys, including those testing entire watersheds by baiting *Phytophthoras* from rivers are routinely performed. To aid the surveying efforts, two general tools are available: a) risk assessment maps, which based on plant susceptibility and climate patterns indicate how favorable an area can be for *P. ramorum*, and b) a wide array of molecular diagnostic tools that will greatly enhance the ability to detect the pathogen. Certainly, no matter how good the diagnostic tools are, it is the sampling scheme that will determine the likelihood of success of detection of the pathogen. Aerial surveys, for instance, only identify dead and dying trees, but cannot identify the subtle symptoms of the most contagious host, California bay laurel.

This problem may be particularly complex for agencies from countries or regions that do not have the pathogen yet and are unaware of the potential susceptibility of their native flora. The

Ministry of Agriculture and Forestry of New Zealand has decided to fund research aimed at determining both the presence of hosts that could be lethally affected and that of hosts that could spread the disease (note that one species could play both roles).

Research at the University of California at Berkeley (UCB) has identified a few hosts of low susceptibility among the New Zealand flora, but also two hosts of high susceptibility. The red southern beech (*Nothofagus fusca*) was extremely susceptible and developed large stem lesions, although it did not support abundant sporulation. The Fuchsia tree (*Fuchsia excorticate*), on the other hand, developed large foliar lesions and was a better substrate for sporulation than Rhododendron. Evidence thus suggests that both types of hosts ("infectious" and "dead-end") exist in New Zealand and that an epidemic could develop in that country. Furthermore, a study of the distribution of these two hosts indicated they coexist abundantly on the west coast of both the North and South islands. Similar approaches aimed at identifying areas containing both epidemiologically-important hosts and highly-susceptible hosts, may be important to identify areas at risk in other regions of the world, and to better focus our surveying schemes.

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XYLELLA FASTIDIOSA AND BACTERIAL LEAF SCORCH OF OAKS: SULIMINAL, SUBTLE, AND SUSPECT

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ABSTRACT

The fastidious xylem-inhabiting bacterium, *Xylella fastidiosa*, is a widely distributed vascular pathogen occurring in a variety of plants and trees. Vectored by several insects (primarily leafhoppers), *X. fastidiosa* causes various symptoms including marginal leaf scorch, decreased fruit production, declining vigor, delayed bud break, stunting, dieback, and sometimes death in susceptible hosts. Surveys have documented a wide distribution of *X. fastidiosa* in oaks, and it is considered by some to be a debilitating pathogen in certain species, especially red oaks. What does all this portend for oak populations? What is the role of *X. fastidiosa* in oak decline? How does *X. fastidiosa* interact with other oak pathogens? This paper briefly reviews the state of our understanding, offers some hopefully relevant commentary, and poses some questions worthy of research attention.

Key words: Insect vectors, leaf disease, tree decline

The fastidious xylem-limited bacterium *Xylella fastidiosa* (Wells et al. 1977) is associated with, and in many cases is considered causal for, leaf scorch/scald and decline diseases in a wide variety of plant species (Hopkins 1977, 1989, Sinclair and Lyon 2005, CABI and EPPO - undated, Mizell et al. undated). With the possible exceptions of Taiwan and India (pending confirmation? – CABI and EPPO - undated), *X. fastidiosa* is known only in the western hemisphere (Global Invasive Species Database 2005, CABI and EPPO - undated,). In the United States, *X. fastidiosa* has been widely reported in association with leaf scorch/leaf scald and decline syndromes on a variety of broad-leaved fruit and shade tree species including members of the genera *Acer*, *Aesculus*, *Carya, Celtis, Cornus, Liquidambar, Morus, Platanus, Prunus, Quercus, Ulmus*, and *Citrus*. Overall, *X. fastidiosa* has been associated with 75-100 species of plants, both woody and non-woody, belonging to some 30 plant families. In many of these host plant species, the bacterium induces no noticeable symptoms of disease (Hopkins 1989, Sinclair and Lyon 2005).

Genetic, pathogenic, nutritional, and host-specificity variation is known within *X. fastidiosa* and unique strains are recognized (Hopkins 1989, Chen et al. 1995, Colletta-Filho et al. 2001, Mehta and Rosato 2001, Schaad et al. 2004, Schuenzel et al. 2005, Zhang et al. 2005, Gould and Lashomb 2006). Several groups of strains have been distinguished within *X. fastidiosa* based on DNA sequences, protein profiles, and host preferences (Schaad et al. 2004, Schuenzel et al. 2005). Schaad et al. (2004) have proposed three identifiable and potentially-useful subspecies, including subspecies *multiplex* which appears unique to elm, sycamore, oak, and maple (Mundell 2005). To date, however, the genus *Xylella* remains monospecific (Sinclair and Lyon 2005, Gould and Lashomb 2006, CABI and EPPO - undated).

X. fastidiosa is an insect-vectored pathogen. According to Purcell (1989), virtually all insects that feed predominantly on xylem fluid are potential vectors of *X. fastidiosa*. Within its known range in North America, the most common insect vectors are leafhoppers (Cicadellidae) in the subfamily Cicadellinae (sharpshooters) and spittle bugs or froghoppers (Cercopidae). Specific vectors vary among host plant species and geographic locations. Extensive lists of known and potential insect vectors are available (Lashomb et al. 2002, Gould and Lashomb 2006, CABI and EPPO - undated, Mizell et al. - undated.).

Following introduction of *X. fastidiosa* into xylem elements of susceptible plants, symptoms of infection develop as the bacterium proliferates in the vascular system (xylem). Symptoms may include marginal leaf tissue necrosis (often in older leaves first), premature leaf abscission, decreased fruit production, decline in vigor, stunting and/or reduced growth, delayed bud break, dieback, and ultimately death (Hopkins 1989, Barnard et al. 1998, Sinclair and Lyon 2005, Gould and Lashomb 2006). This complex of symptoms is consistent with and thought to be largely related to reduced water supply to host plant tissues as the bacterium multiples in xylem elements, although host-produced tyloses and gums, as well as pathogen-produced phytotoxins may be functional in some pathosystems (Hopkins 1989, Sinclair and Lyon 2005, CABI and EPPO - undated).

BACTERIAL LEAF SCORCH OF OAKS

Among the many broad-leaved trees affected by *X. fastidiosa* are several species of oaks (*Quercus* spp.), especially members of the red oak group. Surveys in several eastern states in the U.S. (Chang and Walker 1988, Haygood 1988, Blake 1993, Hartman, Eshenaur and Jarflors 1995, Barnard et al. 1998, Gould et al. 2004) have demonstrated that *X. fastidiosa* is widespread therein and commonly associated with oaks exhibiting leaf scorch and/or decline. Surveys reported from New Jersey (Lashomb et al. 2002, Gould and Lashomb 2006, Gould et al. 2007) suggest that bacterial leaf scorch ("BLS" – the common name of the disease attributed to *X. fastidiosa* infections) is spreading in red oak populations there. Similar to the insect vector situation (above), lists of oaks infected by *X. fastidiosa* are readily available (Lashomb et al. 2002, Sinclair and Lyon 2005, Gould and Lashomb 2006).

CONSIDERATIONS AND UNANSWERED QUESTIONS

Some years ago, this author read a news item in a very popular trade journal that is widely distributed across the U.S. The headline read, "Bacterial Leaf Scorch on the Rise in the Southeast" – a headline clearly intended to signal some level of threat. Such headlines do little to clarify our understanding of this complicated disease scenario. To begin with, the headline presumes at the outset that someone (we) knows (know) what the baseline is; how much BLS did we start with? The reality is that we have no idea, and the "increase" to which the headline refers is likely an increase in the number of reports of BLS resulting from 1) the advent of technologies that facilitate detection and 2) an increase in professional interest and investigation. Of interest to this author is the fact that Dr. George Hepting (one of the "patriarchs" of Forest Pathology in the U.S.) apparently observed oaks exhibiting leaf scorch symptoms decades prior to our ability to detect and identify *X. fastidiosa*. He referred to the symptoms as "leaf dip" (D.H. Marx – personal communication). Recognition of such subtleties and misunderstandings is a must if we are to improve our understanding of BLS in oaks, as well as in other species.

Other factors demanding rigorous evaluation and interpretation when it comes to understanding BLS are environmental considerations and interactions with other diseases. For

example, it is generally recognized that the symptoms resulting from infections by *X. fastidiosa* are "generic" and can be produced by a variety of other causes such as salt damage, drought, other vascular infections, or root disease (Hopkins 1977, Lashomb et al. 2002, Gould and Lashomb 2006). Indeed, Virginia creeper (*Pathenocissus quinquefolia*) inoculated with *X. fastidiosa* failed to express significant leaf scorch symptoms unless subjected to a reduced water (drought?) regime (McElrone, Sherald and Forseth 2001).

How many surveys for or detections of *X. fastidiosa* have considered environmental conditions and have conclusively ruled out the occurrence (simultaneous or sequential) of other diseases? In Florida, for example, turkey oaks (*Quercus laevis*) with and without leaf scorch symptoms and, respectively, with and without detectable infections of *X. fastidiosa* were frequently observed side-by-side. How many of these trees (and which ones) were infected with *Armillaria* and/or *Ganoderma* – common and widespread root pathogens frequently associated with declining turkey oaks (Barnard et al. 1998)? We have no idea. May I submit that this question could be raised in pretty much every situation involving BLS of oaks?

Interestingly, I have read that "scale insects, borers, *Armillaria* root rot, and other biotic diseases may express themselves as secondary pests" on BLS-infected trees (Lashomb et al. 2002, Gould and Lashomb 2006). Do we know that BLS is always primary? Or, could it be secondary (Hopkins 1989)? Why do *X. fastidiosa*-infected and *X. fastidiosa*-free trees of the same species often occur side-by-side (Gould and Lashomb 2006, Barnard et al. 1998)? Is this a function of insect vector preference, genetic variation/resistance in the host, or environmental or pathogenic predisposition to disease development?

What about *X. fastidiosa* and "oak decline"? This phenomenon (oak decline) has been an issue in the southern U.S. for years (Tainter et al. 1990, Oak et al. 2004) and the distribution of oak decline for all intents and purposes can be superimposed on the known range of *X. fastidiosa*, and vice versa. Could there be a link? To this author's knowledge, there has never been a serious attempt to find out.

There is much we do not know about *X. fastidiosa* and the various diseases with which it is associated. Statements by D. L. Hopkins (1989) perhaps state things best.

- "The combined list of natural hosts for all strains of *X. fastidiosa* ` evidently is limited more by the effort spent in the search for alternate hosts than by the actual host specificity of the bacterium."
- "Except for a few host-pathogen combinations like PD of grapevine and phony disease of peach, *X. fastidiosa* could be considered a weak or opportunistic pathogen. Strains of *X. fastidiosa* often appear to survive as residents of the xylem vessels in symptomless hosts, but accumulate and produce disease symptoms only if the host is weakened by some other stress factor. …Stress factors favoring *X. fastidiosa* diseases include drought, other diseases, root pruning with cultivation equipment, overproduction of fruit, normal fruit maturation, and senescence. In most hosts, symptoms of the diseases are not visible until either the time of fruit maturation or late autumn when the hosts are senescing."
- "In addition to senescence apparently affecting susceptibility of hosts to *X. fastidiosa*, symptoms of the diseases – chlorosis, abscission of leaves, and acropetal symptom development – are also characteristic of plant senescence."

- "…host senescence appears to be fundamental in diseases caused by *X. fastidiosa, …"*
- "While other stress factors on the host may favor *X. fastidiosa*, chronic, nonlethal infection by the bacteria also may predispose its hosts to other pathogens and stresses. This seems especially to happen with the shade tree diseases."
- "With many *X. fastidiosa*-associated diseases, it is difficult to determine whether the bacterium is the primary or a secondary stress factor. In some cases, a synergism with another pathogen or stress factor may be required for disease development."

Given the complexities and sometimes conflicting realities, it is clear that a thorough understanding of the role of *X. fastidiosa* in scorched and/or declining oaks demands more investigation. Further, assuming climate change is inevitable (and I would hazard a guess that it is – one way or another), the nuances of pathogen X insect vector X environment interactions seem endless. Careful research and data interpretation are essential.

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