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 thinnervalled 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 Glaucioideae) 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. fusiformis* 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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**LITERATURE CITED**


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 sapropes, 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 *Thielaviopsis* 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. moniliformis* 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 site 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. fimбриata 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 HaeIII 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 PstI-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.
LITERATURE CITED


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, 818 characters were constant, 138 characters were parsimony uninformative, 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 km² (50 mi²) 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.

LITERATURE CITED


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

<table>
<thead>
<tr>
<th>Cause</th>
<th>No. Centers</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
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<td>69</td>
</tr>
<tr>
<td>Military Ops</td>
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<td>0.8</td>
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<td>8</td>
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</tr>
<tr>
<td>Brush Piles</td>
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<td>19.3</td>
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<tr>
<td>Blow down</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Fire</td>
<td>4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

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 Pseudopityophthorus 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 Eggers 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. ellipsoidalis* 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 Moltzanz, 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.

**ACKNOWLEDGEMENTS**

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**LITERATURE CITED**


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 Phytophthora 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 2B).

**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.

ACKNOWLEDGEMENTS

The authors would like to thank the following individuals: From the USDA Forest Service, Forest Health Protection: Joseph O’Brien and Dale Starkey for their pathology and forestry expertise as well as their administrative and technical support. From the USDA Forest Service, North Central Research Station, Forest Disease Unit: Kathy Kromroy for her intellectual contributions during the conceptualization and execution of the project; Kathy Ward and Paul Castillo for their technical assistance in collecting oak wilt field data and the acquisition of appropriate data layers for use in the analysis. From the U.S. Army III Corps and Ft. Hood Program: John Cornelius (Director for Endangered Species Management) and Charles Pekins for their technical and field support. From the DPW ENV Natural Resources Branch, Fort Hood, TX: Steve Jester for site characterization. From The Nature Conservancy, Texas Chapter, Fort Hood Project: Sheila Jackson for field work. Texas A&M University, Department of Plant Pathology and Microbiology: Coy Crane and Olivie Schill for field work. From the Minnesota Department of Natural Resources, Division of Forestry: Susan Burks for her participation as a stakeholder.

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http://www.texasoakwilt.org/Gallery/maps/Intensity_Map.jpg

http://www.fs.fed.us/foresthealth/technology/spatialstatistics/index.shtml

http://www.fs.fed.us/foresthealth/technology/spatialstatistics/index.shtml
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:

<table>
<thead>
<tr>
<th>Anoka County Dependent Variable Sample</th>
<th>Fort Hood Dependent Variable Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Point Theme</strong></td>
<td><strong>Point Theme</strong></td>
</tr>
<tr>
<td>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.</td>
<td>A. Field visits by the USDA FS Forest Health Technology Enterprise Team (FHTET), Region 8, Texas A &amp; M and Texas Department of Forestry personnel, to randomly select “healthy and “active” oak wilt sites.</td>
</tr>
<tr>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>C. Field visits by the USDA FS NCRS Forest Disease Unit and FHTET to randomly selected healthy oak forest sites.</td>
<td></td>
</tr>
</tbody>
</table>
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)**

<table>
<thead>
<tr>
<th>A.</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.</td>
<td>Distance to Lakes: USGS 1:100,000 DLG data, measured using ArcView Spatial Analyst, distance in meters from feature function.</td>
</tr>
<tr>
<td>C.</td>
<td>Distance to Streams: USGS 1:100,000 DLG data, measured using ArcView Spatial Analyst, distance in meters from line feature function.</td>
</tr>
<tr>
<td>D.</td>
<td>Drainage: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); <a href="http://soildatamart.nrcs.usda.gov">http://soildatamart.nrcs.usda.gov</a></td>
</tr>
<tr>
<td>E.</td>
<td>Elevation: derived from the USGS 30 m (1:24000) DEM.</td>
</tr>
<tr>
<td>F.</td>
<td>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).</td>
</tr>
<tr>
<td>I.</td>
<td>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.</td>
</tr>
<tr>
<td>J.</td>
<td>Slope degrees: derived from the USGS DEM using ArcView Spatial Analyst (ESRI) slope function</td>
</tr>
<tr>
<td>K.</td>
<td>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.</td>
</tr>
</tbody>
</table>

**Fort Hood Independent Variables (L-Z)**

<table>
<thead>
<tr>
<th>L.</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.</td>
<td>Elevation derived from the USGS 30 meter resolution DEM (1:24000 scale).</td>
</tr>
<tr>
<td>P.</td>
<td>Forb percent: from USDA, NRCS, SSURGO Data Version 2.1 (December 2003); <a href="http://soildatamart.nrcs.usda.gov">http://soildatamart.nrcs.usda.gov</a></td>
</tr>
<tr>
<td>Q.</td>
<td>Landcover: Derived from Landsat Imagery collected 5/16/2003; a = open, b = coniferous, c = deciduous.</td>
</tr>
<tr>
<td>R.</td>
<td>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).</td>
</tr>
</tbody>
</table>
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.

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

Table 4. Misclassification error rates for each study area:

<table>
<thead>
<tr>
<th></th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Non-Urban</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classified Absent</td>
<td>96</td>
<td>24</td>
</tr>
<tr>
<td>Classified Present</td>
<td>13</td>
<td>123</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. Urban</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classified Absent</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>Classified Present</td>
<td>8</td>
<td>157</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Absent</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Fort Hood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classified Absent</td>
<td>271</td>
<td>18</td>
</tr>
<tr>
<td>Classified Present</td>
<td>7</td>
<td>88</td>
</tr>
</tbody>
</table>
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.
b

Anoka County Non-Urban Model

- Population Change < 76
  - Silt < 1.1
    - Landsat Band 3 September < 13.5
      - Landsat Band 7 May < 23.5
        - Landsat Band 5 May < 81.5
      - Landform < 0.05
        - Population Change < 9.5
          - Slope < 0.73
  - Organic Matter < 1
    - Population
      - < 397.5
      - < 76
  - May < 33.5
      - Population Change < 1
        - < 9.5
          - Slope < 0.73
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 effects of 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 duddled 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 m²/ha. Two to 4 years after passage of the disease front, total basal area had been reduced to 4 m²/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.

**LITERATURE CITED**


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.
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](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](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 than 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.

ACKNOWLEDGEMENTS
This work was supported by grants from the Texas Nature Conservancy and from the USDA Forest Service, Forest Health Technology Enterprise Team, Ft. Collins, CO. The authors would like to thank Marla Downing, USDA Forest Service, Vernon Thomas of INTECS International Inc., and Robin Reich from Colorado State University for technical assistance and surveying plots. We would also like to thank the technicians with the Department of Plant Pathology and Microbiology, Texas A&M University, Sheila Jackson with the Nature Conservancy at Fort
Hood, and Dale Starkey, USDA Forest Service, Forest Health Protection in Pineville, LA, for their support. John Cornelius with the Environmental Resources Branch and Fort Hood provided valuable information on the biology and habitat requirements of the GCW. We also thank Steve Jester and Tom Greene with the Fort Hood Nature Conservancy office for their support, guidance, and information during the course of the study.

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

<table>
<thead>
<tr>
<th>Category(^a)</th>
<th>Tree /Hectare</th>
<th>% Juniper</th>
<th>% Hardwood</th>
<th>J:O Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCW/non-OW</td>
<td>886</td>
<td>77</td>
<td>23</td>
<td>6.57:1</td>
</tr>
<tr>
<td>GCW/OW</td>
<td>639</td>
<td>56</td>
<td>44</td>
<td>1.66:1</td>
</tr>
<tr>
<td>Non GCW/non-OW</td>
<td>90</td>
<td>45</td>
<td>54</td>
<td>2.48:1</td>
</tr>
<tr>
<td>Non GCW OW</td>
<td>570</td>
<td>14</td>
<td>86</td>
<td>0.24:1</td>
</tr>
<tr>
<td>GCW/NS</td>
<td>1298</td>
<td>65</td>
<td>35</td>
<td>3.16:1</td>
</tr>
</tbody>
</table>

\(^a\)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.

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

\(^b\)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.

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

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 *Colopterus* 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.

**LITERATURE CITED**


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

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

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.

<table>
<thead>
<tr>
<th>Tree stand type</th>
<th>Days after wounding</th>
<th>Number of beetles</th>
<th>Assayed with Cf&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Assayed</td>
</tr>
<tr>
<td>Live oak</td>
<td>1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Red oak</td>
<td>1</td>
<td>109</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of beetles contaminated with *Ceratocystis fagacearum*.

<sup>b</sup> 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Langford</th>
<th>Solana</th>
<th>Johnson</th>
<th>TNLA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Colopterus truncatus</em></td>
<td>27</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Colopterus maculatus</em></td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><em>Cryptarcha concinnus</em></td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>Carpophilus spp.</em></td>
<td>28</td>
<td>40</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 1. Number collected and frequency of *Ceratocystis fagacearum* (Cf) isolated from three *Colopterus* spp. (*Co. truncatus*, *Co. niger*, and *Co. semitecutus*) 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.

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, Kurdyka and Lewis 1990, Ambourn, Juzwik and Moon 2005). From an epidemiological perspective, the initiation of new disease centers by nitidulids is a critical stage in the oak wilt disease cycle. This means of spread is also a controversial issue for arborists throughout the range of oak wilt because pruning wounds are implicated as important infection courts for nitidulids in the oak wilt syndrome. Much of the controversy involves the recommended oak wilt control measure of applying wound dressings to prevent nitidulids from inoculating pruning cuts on susceptible trees.

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

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

Due to the requirement of fresh wounds for infection by the oak wilt pathogen, wound treatments have long been a potential control measure of interest to researchers and practitioners.
(Drake, Kuntz and Riker 1958, Gibbs 1980). Juzwik, French and Juzwik (1985) wounded over 5,000 trees to study natural infection of oaks with *C. fagacearum*. In Minnesota, infection from wounding occurred from May to mid-June. Numerous wounds were treated with a variety of commercially-available wound dressings including Leonard’s Tree Compound (A.M. Leonard and Sons, Inc. Piqua, OH), Cabots Tree Healing Paint (Samuel Cabot Mfg., Inc., Boston, MA), and Treekote (Walter C. Clark and Son, Orange, CT). Of the 322 wounded trees treated with wound dressings, none of the trees became infected, nor did any unwounded trees contract the disease. Infection rates on untreated, wounded trees in different plots varied from 3% to 29%, depending on location and time of year the tree was wounded. As a result of these and other related studies, most educational materials developed by state and federal agencies include wound paints as part of comprehensive oak wilt control programs (Appel et al. 1995, O’Brien et al. 1999, French and Juzwik 1999, Bonello 2001, Cummings-Carlson and Martin 2005).

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

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

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

Pruning branches with aspect ratios lower than the predicted ratio (0.39) resulted in relatively small amounts of discolored wood. In a related study, Eisner et al. (2002) demonstrated that branches with lower aspect ratios, no pith connections, and visible branch collars had lower
conductivity ratios, which means that these features are associated with restricted movement of water from the parent stem to the branch. They found that lower conductivity ratios result in a decrease in discoloration, and this restriction in water flow may also reduce the infection potential of *C. fagacearum*. Studies measuring the impact of proper pruning to limit disease transmission must ensure that true branches, rather than codominant stems which do not have branch collars or BPZs, are utilized in order to accurately assess the benefits of these inherent morphological features.

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

**MATERIALS AND METHODS**

The study was conducted on an oak-woodland ranch north of Austin, TX, located at -97°45’12”W and 30°28’23”N. The tree species on the ranch consisted mainly of live oaks (*Q. fusiformis*), but cedar elm (*Ulmus crassifolia*) and gum bumelia (*Bumelia 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
had the next highest infection level (60%) and were also significantly greater than the negative control treatments (P=0.019). Painting the flush cuts (treatment IV) reduced the infection level to 20%, which made that treatment significantly less than the positive controls (P=0.024). On one of these trees that became infected, the paint was not completely dry and slipped off the wound, partially exposing the cut surface of the branch, when the spore inoculation was brushed onto the wound. This tree was immediately resprayed with pruning paint and allowed to dry before reapplication. Of the trees that were treated with the Shigo cuts (treatment V), 40% became infected as shown in Table 1. There were no significance differences among the non-flush cut treatments and the other treatments.

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

ACKNOWLEDGEMENTS

The use of trade names in this publication does not imply endorsement by the authors or Texas A&M University of the products named, nor criticism of similar ones not mentioned. We acknowledge the technical assistance of the following Texas A&M University collaborators: Mr. Thomas Kurdyla, technician, and Mr. Jeff Lehde, graduate student.

LITERATURE CITED


Aufess, H. Von. 1975. The formation of a protective zone at the base of branches of broad-leaved and coniferous trees and its effectiveness in preventing fungi from penetrating into the heartwood of living trees. Forstwissenschaftliches Centralblatt 94: 140-152.


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

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

* 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.

<table>
<thead>
<tr>
<th>Treatments (No.)</th>
<th>Mean DBH (cm)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control (I)</td>
<td>17.89</td>
<td>0.4792</td>
</tr>
<tr>
<td>Negative control (II)</td>
<td>17.52</td>
<td>0.8942</td>
</tr>
<tr>
<td>Flush cut painted (IV)</td>
<td>20.70</td>
<td>1.1187</td>
</tr>
<tr>
<td>Flush cut unpainted (III)</td>
<td>20.06</td>
<td>1.0136</td>
</tr>
<tr>
<td>Shigo cut (V)</td>
<td>22.26</td>
<td>1.0804</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Treatments (No.)</th>
<th>Stem aspect ratios</th>
<th>Mean</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2-0.29</td>
<td>0.3-0.39</td>
<td>0.4-0.49</td>
</tr>
<tr>
<td>Flush cut unpainted (III)</td>
<td>1/1</td>
<td>1/2</td>
<td>3/6</td>
</tr>
<tr>
<td>Flush cut painted (IV)</td>
<td>0/1</td>
<td>0/4</td>
<td>2/5</td>
</tr>
<tr>
<td>Shigo cut (V)</td>
<td>0/1</td>
<td>3/5</td>
<td>1/4</td>
</tr>
<tr>
<td>Total</td>
<td>1/3</td>
<td>4/11</td>
<td>6/15</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Treatments (No.)</th>
<th>Range</th>
<th>Mean wound diameter (cm)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flush cut unpainted (III)</td>
<td>4.1 – 17.0</td>
<td>7.8a</td>
<td>1.3568</td>
</tr>
<tr>
<td>Flush cut painted (IV)</td>
<td>4.8 – 8.1</td>
<td>6.6ab</td>
<td>0.3908</td>
</tr>
<tr>
<td>Shigo cut (V)</td>
<td>2.8 – 8.1</td>
<td>5.3b</td>
<td>0.5283</td>
</tr>
</tbody>
</table>

*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 (Treatment 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 4-6°C 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 -80°C 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 -80°C.

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 26°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: $10^3+10^3$” 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.

**ACKNOWLEDGEMENTS**

We wish to thank Mario Mandujano and Jim Klug for their technical assistance in our work.

**LITERATURE CITED**


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.

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

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Seedlings inoculated with water</td>
</tr>
<tr>
<td>PM447 only</td>
<td>Seedlings inoculated with the hypovirulent, PM447 strain</td>
</tr>
<tr>
<td>Wild-type only</td>
<td>Seedlings inoculated with one wild-type strain (Fenn, Westcott, or Beal)</td>
</tr>
<tr>
<td>Challenge: 0 weeks</td>
<td>Seedlings co-inoculated with PM447 and one wild-type strain (Fenn, Westcott, or Beal)</td>
</tr>
<tr>
<td>Challenge: 1 week</td>
<td>Seedlings inoculated with PM447, then challenge inoculated 1 week later with a wild-type strain (Fenn, Westcott, or Beal)</td>
</tr>
<tr>
<td>Challenge: 3 weeks</td>
<td>Seedlings inoculated with PM447, then challenged 3 weeks later with a wild-type strain (Fenn, Westcott, or Beal)</td>
</tr>
<tr>
<td>Challenge: 4 weeks</td>
<td>Seedlings inoculated with PM447, then challenged 4 weeks later with a wild-type strain (Fenn, Westcott, or Beal)</td>
</tr>
</tbody>
</table>
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.

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

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Trees were inoculated with water</td>
</tr>
<tr>
<td>PM447</td>
<td>Trees were inoculated with the hypovirulent strain, PM447</td>
</tr>
<tr>
<td>Westcott</td>
<td>Trees were inoculated with the wild-type strain, Westcott</td>
</tr>
<tr>
<td>Challenge: 1 week</td>
<td>Trees were inoculated with PM447, then challenge inoculated</td>
</tr>
<tr>
<td></td>
<td>with Westcott one week later</td>
</tr>
<tr>
<td>Challenge: 2 weeks</td>
<td>Trees were inoculated with PM447, then challenge inoculated</td>
</tr>
<tr>
<td></td>
<td>with Westcott two weeks later</td>
</tr>
<tr>
<td>Challenge: 3 weeks</td>
<td>Trees were inoculated with PM447, then challenge inoculated</td>
</tr>
<tr>
<td></td>
<td>with Westcott three weeks later</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average DR: 2005</th>
<th>Average DR: 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PM447</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Westcott</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Challenge: 1 week</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Challenge: 2 weeks</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Challenge: 3 weeks</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Average DR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Trees were inoculated with water</td>
<td>0 a</td>
</tr>
<tr>
<td>Westcott</td>
<td>One tree was inoculated with the wild-type strain, Westcott</td>
<td>2 b</td>
</tr>
<tr>
<td>PM447: 10^1</td>
<td>Trees were inoculated with 10^1 spores of PM447</td>
<td>0 a</td>
</tr>
<tr>
<td>PM447: 10^2</td>
<td>Trees were inoculated with 10^2 spores of PM447</td>
<td>0 a</td>
</tr>
<tr>
<td>PM447: 10^3</td>
<td>Trees were inoculated with 10^3 spores of PM447</td>
<td>0.5 a</td>
</tr>
<tr>
<td>PM447: 10^5</td>
<td>Trees were inoculated with 10^5 spores of PM447</td>
<td>2 b</td>
</tr>
<tr>
<td>PM447: 10^1+10^1</td>
<td>Trees were inoculated with 10^1 spores of PM447, then were inoculated two weeks later with 10^1 spores of PM447</td>
<td>0.33 a</td>
</tr>
<tr>
<td>PM447: 10^2+10^2</td>
<td>Trees were inoculated with 10^2 spores of PM447, then were inoculated two weeks later with 10^2 spores of PM447</td>
<td>1 b</td>
</tr>
<tr>
<td>PM447: 10^3+10^3</td>
<td>Trees were inoculated with 10^3 spores of PM447, then were inoculated two weeks later with 10^3 spores of PM447</td>
<td>1 b</td>
</tr>
<tr>
<td>PM447: 10^1+10^1+10^1</td>
<td>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</td>
<td>1.67 b</td>
</tr>
<tr>
<td>PM447: 10^2+10^2+10^2</td>
<td>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</td>
<td>1.33 b</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Average DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Trees were inoculated with water</td>
<td>0</td>
</tr>
<tr>
<td>PM447: $10^1$</td>
<td>Trees were inoculated with $10^1$ spores of PM447</td>
<td>1</td>
</tr>
<tr>
<td>PM447: $10^3$</td>
<td>Trees were inoculated with $10^3$ spores of PM447</td>
<td>2</td>
</tr>
<tr>
<td>PM447: $10^5$</td>
<td>Trees were inoculated with $10^5$ spores of PM447</td>
<td>2</td>
</tr>
<tr>
<td>PM447: $10^1+10^1$</td>
<td>Trees were inoculated with $10^1$ spores of PM447, then</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>inoculated a second time with $10^1$ spores of PM447 two weeks later</td>
<td></td>
</tr>
<tr>
<td>PM447: $10^1+10^3$</td>
<td>Trees were inoculated with $10^1$ spores of PM447, then</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>inoculated a second time with $10^3$ spores of PM447 two weeks later</td>
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</tr>
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<td>PM447: $10^3+10^3$</td>
<td>Trees were inoculated with $10^3$ spores of PM447, then</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>inoculated a second time with $10^3$ spores of PM447 two weeks later</td>
<td></td>
</tr>
<tr>
<td>Westcott</td>
<td>Trees were inoculated with the wild-type strain, Westcott</td>
<td>2</td>
</tr>
<tr>
<td>Challenge: $10^1$</td>
<td>Trees were inoculated with $10^1$ spores of PM447, then</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>challenged two weeks later with Westcott</td>
<td></td>
</tr>
<tr>
<td>Challenge: $10^3$</td>
<td>Trees were inoculated with $10^3$ spores of PM447, then</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>challenged two weeks later with Westcott</td>
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<td>2</td>
</tr>
<tr>
<td></td>
<td>challenged two weeks later with Westcott</td>
<td></td>
</tr>
<tr>
<td>Challenge: $10^1+10^1$</td>
<td>Trees were inoculated PM447: $10^1+10^1$ as described</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>above, then challenged with Westcott two weeks later</td>
<td></td>
</tr>
<tr>
<td>Challenge: $10^1+10^3$</td>
<td>Trees were inoculated PM447: $10^1+10^3$ as described</td>
<td>0.67</td>
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<td>above, then challenged with Westcott two weeks later</td>
<td></td>
</tr>
</tbody>
</table>
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.