‘Future Direction of USDA Forest Service Research

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The USDA Forest Service has been involved in Texas oak wilt research since 1976. Despite research successes, there are still many important research areas that have not been addressed or sufficiently investigated to answer the key questions required for making sound disease management decisions. Some of the priority areas planned for future research by the Southern Hardwoods Laboratory include; epidemiology, reforestation (regeneration), chemical control, biological control, trenching, disease physiology, host resistance, sanitation, and early diagnosis. Each of these topics will be prioritized based on present and expected future management needs and categorized into short-term and long-term study plans for achieving research goals. Details of research emphasis are presented for some of these topics that will be addressed in future oak wilt research by the Southern Station.

INTRODUCTION

The Southern Hardwoods Laboratory (SHL) at Stoneville, MS became involved in Texas oak wilt research in late 1976. Robert Lewis, the first USDA Forest Service pathologist from the SHL to study oak wilt, successfully isolated and identified the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, from live oaks ***(Quercus virginiana*** Mill.) and red oaks (Q. *texana* Buckl.) in central Texas in early 1977 (Lewis 1977). This diagnosis came 16 years after the first diagnosis of oak wilt in Texas (near Dallas) by Oscar Dooling (1961), and 43 years after a serious live oak disease of unknown etiology, now presumed to be oak wilt based on symptomology, was described by Taubenhaus in Austin (1934, 1935).

Subsequent work by Lewis (1979b, 1981b) further clarified that the deaths of most oaks in the Hill Country of Texas were due to the oak wilt fungus alone and not to an oak decline disease complex. Nevertheless, the disease continued to be called oak decline for several years. Lewis later worked on’chemical control (Lewis 1979a, Lewis and Brook 1985), disease loss assessment and prevention (Lewis 1979b, Lewis 1981b, Lewis and Oliveria 1979, Lewis et al. 1983), epidemiology (Lewis 1984), effects of temperature on C. *fagacearum* in trees (Lewis 1981a, 1985c), survival of the fungus in living and dead trees (Lewis 1985a, 1985b, 1987), and insect vectors (Lewis 1983) before he was promoted to Assistant Director and transferred to the Northeastern Forest Experiment Station in 1986. Oak wilt work at the SHL continued when Ted Filer took over the project in July 1986. His work dealt mostly with the testing of systemic fungicides (Filer 1985, 1986b. 1990b, Filer and Smyly 1992) and endophytic bacteria (Filer 1986a, 1987, 1988, 1989, 1990a) for oak wilt control.

Oak wilt disease annually kills thousands of live oaks in at least 46 counties of Texas. Live oaks are valued for their aesthetics in urban and suburban areas and for the shade they provide for livestock in rural areas. Consequently, live oaks contribute significantly to real estate property values in the state. The impact of the disease on property values in Texas prompted the founding and organization of the Texas Oak Wilt Demonstration Project in 1982 that became the Texas Oak Wilt Suppression Project (TOWSP) in 1988, administered by the ‘Texas Forest Service (TFS). The SHL became committed to cooperative planning and activities with the TOWSP for oak wilt control in late 1991 with the SHL having a primary research role. The present emphasis of the SHL is to continue to work collaboratively with the TOWSP, provided that funding and support continues, to identify the most promising research areas that may lead to more effective control methods.

The future emphasis of SHL oak wilt research will include basic research of oak wilt epidemiology in Texas live oaks, host-parasite physiology, mode of action of fungicides, and applied research to improve present control methods and to evaluate new methods and approaches to disease control. The remainder of this paper will be devoted to a more detailed discussion of some key areas that will be emphasized in future oak wilt research by the SHL.

BASIC RESEARCH OF HOST-PARASITE INTERACTIONS

Epidemiology

A better understanding of oak wilt epidemiology in Texas live oaks is among the. more important priority areas for basic research. The factors contributing to differences between oak wilt epidemiology in Texas live oaks and deciduous oaks in mixed hardwood forests of Northern and Eastern States and between live oak and red oak infection centers in Texas should be identified. The differences in host species, growth form (e.g., clumped clonal stands called "motts" in live oaks), climatic conditions, edaphic factors, rainfall patterns, vectors, and pathogen genetics no doubt have significant impacts on disease progression. Some of these questions are being investigated in cooperative studies with Texas A&M University.

Studies to clarify the importance of root transmission in oak wilt epidemiology are needed. It is particularly important to determine the rate that C. *fagacearum* moves through interconnected live oak roots. The often rapid rate of expansion of infection centers indicates that the fungus may spread more rapidly through live oak stands than through oak species, in Northern and Eastern States. The possibility that the fungus and its enzymes and toxin(s) may be spreading far in advance of symptomatic trees at the edge of infection centers could be complicating attempts to control root transmission by trenching.

Determining the capacity of the oak wilt fungus to survive in root systems at different stages of disease development and following the death of trees is equally important. The ability of the fungus to survive saprophytically in dead roots or in soil will determine whether regeneration with live oak within infection centers is practical. Such determinations will require some host studies such as determining rates of root death following death of crowns, frequencies of root connections and grafting among live oaks and red oaks, and rate of regrafting and regeneration of severed roots after trenching. Studies in cooperation with Dr. David Appel at Texas A&M University are in progress to determine the effects of host characteristics on root transmission.

Determining the primary means by which the pathogen spreads above ground will greatly improve our basic understanding of long-distance dispersal and of mechanisms by which new infection centers appear. This knowledge will lead to more appropriate control measures to reduce development of new infection foci. The rate of occurrence of new infection centers probably contributes to the increase in land area affected by oak wilt more rapidly than does the spread of the fungus through root grafts. This hypothesis is likely true because the generation of new infection centers allows concomitant development of multiple infection centers that more efficiently cover land area than expansion of a single infection center that is limited by random root grafting and interconnected roots between individual trees and by the clustering of trees in motts.

The identification of inoculum sources and the importance of inoculum types also is necessary for an adequate understanding of epidemiology. For example, the relative importance of infected red oaks with fungal mats, infested firewood, vectors, and tree wounding in oak wilt epidemiology must be known. Presently, C. *fagacearum* is known to produce fungal mats only on red oaks in Texas. Consequently, red oaks are commonly believed to be the major source of inoculum from which insect vectors bring inoculum to healthy live oaks. However, if wood-boring insect vectors utilize live oak firewood for food or brooding sites, the mycelium in infected live oak wood could be a potential source of inoculum. The role of red oaks deserves particular attention because these trees, although generally mote susceptible to oak wilt, have been observed to be the last trees to become infected and die within some infection centers. Other reservoirs of inoculum also could exist.

If firewood was an important source of inoculum, then a state quarantine to restrict intercounty transport of firewood could be useful even if such a quarantine could not be strictly enforced. The question of whether wounds are always required for introduction of inoculum by vectors is intriguing. It is reasonable to suggest that some wood-boring insects capable of boring into sapwood without the aid of wounds might be vectors of C. *fagacearum.* Finally, the types of inoculum capable of infecting healthy trees, the inoculum potential required, and the importance of inoculum types should be examined. Are conidia and ascospores the only important inoculum types in nature, or can mycelium also be moved by vectors to healthy trees?

Host-Parasite Physiology

Another important area for basic research is the host-pathogen interaction associated with pathogenesis and its effects on host physiology. What are the predisposing environmental factors that increase a tree's susceptibility to infection? The 'water balance of challenged trees is invariably an important factor in a vascular wilt disease, but predisposing factors such as wounding, plant nutrition, carbon balance, and degree of root connections with adjacent trees could all have a profound influence on disease development and symptom expression. For example, carbon balance can have a large influence on energy reserves needed for host defense mechanisms. Investigations of these factors could provide answers to new disease management approaches that alleviate symptoms, delay pathogenesis, and prolong tree survival providing more time for host resistance responses to become effective.

A detailed understanding of the mechanism(s) of disease development would be valuable in many aspects of presymptomatic disease diagnosis, disease management, and post suppression (control) evaluations. The roles of enzyme systems in vascular plugging and of toxin(s) in water loss and defoliation are important aspects of pathogenesis that warrant further study.

Effects of Systemic Fungicides on Host Physiology

The common use of systemic triazoles, ergosterol-inhibiting fungicides such as propiconazole that have side effects on phytohormone activity, may complicate post treatment evaluations. These materials tend to have a greening effect on live oak foliage that may be giving a false sense of security. The temporary greening of leaves on infected live oaks may be simply a cosmetic solution that masks symptom development for a time until the trees suddenly defoliate and die. A thorough study of the effects of triazoles on live oak physiology would provide useful insights toward our understanding of their modes of action with regard to host physiology as well as our ability to better interpret the symptomology of treated trees.

APPLIED RESEARCH FOR CONTROL OF OAK WILT

Early Diagnosis

The ability to determine the extent and rate of spread of fungal inoculum or toxic metabolites from diseased (source) trees within infection centers to asymptomatic trees at the edge of infection centers is the most challenging problems associated with oak wilt suppression in Texas. The difficulty of determining which trees harbor the fungus has led to recognition of the need for a diagnostic tool that will detect the presence of *C. fagacearum,* or its metabolites, in trees prior to symptom development.

An early detection method for applied (control) applications could be used to identify the presence of newly forming infection centers with suspect trees either known to be predisposed to infection or in very early stages of symptom development. Detecting infection at an earlier point in disease development would facilitate efforts to contain the infection early and possibly eradicate new infection centers before they become well established. Eradicating a young infection center should require much less effort than eradicating an older, more developed center. Consequently; early detection methods would allow determinations of the proper placement of control treatments, such as trenches, in relation to infection centers. Early evaluations of control treatment effectiveness also should be possible. These evaluations would be useful in identifying situations where secondary, follow-up treatments are needed.

Methods for early diagnosis could be used as effective research tools as well. Knowledge of tree infection status would be extremely useful for establishing adequate check treatments and for confidently assessing control treatments and the effects of fungicides and toxins on host physiology. Diagnostic tools for early detection also might be applied in epidemiology studies, particularly in the identification of the mechanisms and nature of vegetative spread by root grafting and common root systems.

Dr. Garry Cole, University of Texas at Austin, is developing an antigen detection kit based on serological recognition of a 35 kilodalton protein in xylem sap for routine field diagnoses of presymptomatic trees (Silverman et al., this proceedings). We plan to participate in field testing of this detection kit as soon as it becomes available. Remote sensing at low altitudes or from the ground with digital infrared thermometers is another diagnostic tool that could be further developed and evaluated.

Short-term Control

Trenching continues to be the preferred primary control method to reduce root transmission of the oak wilt fungus. However, there is considerable concern about its long-term effectiveness since trench failures are common. Breakouts along trenches have been attributed to improper trench placement, inadequate trench depth, incomplete trenches, and regrowth across trenches. TOWSP personnel of the TFS have indicated that the effectiveness of their suppression trenches have improved from about 80 percent failure rate, since the beginning of their record keeping in 1988, to approximately 20 percent failure rate at present (Gehring, these proceedings). The causes of trench failures, the effective life (longevity) of trenches, and the rate of regrowth and grafting of roots in trenches are all important subjects for future research. Further work to identify other possible methods to reduce root transmission should be examined.

The applications of systemic fungicides, often in combination with trenches, similarly have met with mixed results. Success with injections of propiconazole into root flares has been sporadic, although propiconazole does appear to provide long-term protection of some trees by preventing or delaying crown symptoms. A major question with fungicides is whether or not present methods of applying these systemic materials are providing adequate coverage of infected tissues, especially root tissues. Root inoculum is particularly important in disease progression when infection is initiated by root transmission since the fungus first enters and accumulates most of its inoculum potential in the roots. Consequently, if most of the injected fungicides are translocated upward through xylem into the crown, the majority of the inoculum in the roots would not be treated.

Future research is needed to quantify' fungicide levels and distributions within trees following injections with different injection methods to determine if adequate coverage of infected tissues is being achieved. An insufficient accumulation of fungicide in the roots could explain much of the frustration and inconsistencies associated with fungicide injections. In addition, research is needed to further evaluate the potential for using systemic fungicides for prophylactic (preventative) and therapeutic (curative) applications.

Long-term Control

Attempts to achieve long-term (permanent) control of oak wilt in treated trees will require more intensive research of new approaches in biological control, host resistance, reforestation, and more advanced approaches to permanently modify the genetics of host or pathogen using biotechnology. Endophytic microbes with systemic' capabilities 'that produce' specific antifungal metabolites would be particularly good candidates as biocontrol agents of oak wilt in live oaks because the interconnected, grafted, or common root systems could facilitate dispersal of systemic endophytes throughout motts, eliminating the need to inoculate every tree. Host resistance or tolerance has been largely neglected in oak wilt research.

Studies to better evaluate the possibilities for breeding, hybridization, and grafting of oak species for resistance should be investigated (see Greene, these proceedings). If different tree species could be considered acceptable replacements for live oaks, then reforestation with nonhost (immune) species would be a viable long-term solution particularly in devastated areas where most live oaks already have been removed. Biotechnology approaches to disease control through' gene manipulations could provide future solutions if knowledge of gene regulation in this field continues to progress.

CONCLUSIONS

Oak wilt disease in Texas, although caused by the same organism as in northern and eastern regions of the United States, can be distinguished from the disease manifested in oaks of Midwestern and Eastern States. The disease appears to progress more rapidly in many cases in Texas live oaks than in northern oak species. The differences in disease expression could be due to differences in host-pathogen interactions, host resistance, virulence of geographical races of the, fungus, or in environmental influences, on disease expression. The Texas Hill Country has a much more xeric environment than the Northern and Eastern States.

The average daily temperature and atmospheric vapor pressure deficit (evaporative potential) are significantly greater in central Texas than in Northern States. The soils are often shallow and rocky with heavy clay content. All of these factors contribute toward exacerbating drought stress conditions in normal years. Trees growing under xeric conditions tend to be more susceptible (predisposed) to vascular wilt diseases because their water balance is often in deficit, particularly during hot, dry, summer months.

Live oak is a quite unique host of C. *fagacearum,* different in many ways from typical hosts. This species often grows in clumped stands called "motts" that tend to share common root systems and may essentially behave as one organism, having a closely shared physiological interaction. They are evergreen oaks that maintain some physiological activity throughout the year, probably allowing the fungus to sustain some metabolic activity throughout the year as well. Stands of live oaks are typically very close tomonocultures with relatively few other species present to breakup community structure unlike the mixed hardwood stands in the northern and eastern United States. Live oaks also produce relatively shallow root systems due to limited soil depth. This facilitates development of root diseases.

These differences suggest that studies of oak wilt in Texas will likely require some modifications of established research methods and variations in disease management approaches from those used in other regions of the United States where *C. fagacearum* is endemic. An integrated approach to control likely will be required since many factors can influence pathogen spread and disease development. Finally, the variability associated with oak wilt disease, host characteristics, and environmental parameters in different live oak habitats of Texas (urban and rural situations) will probably require different approaches to disease management.

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