Oak Wilt Perspectives:

The Proceedings of the National Oak Wilt Symposium

June 22-25, 1992
Austin, Texas

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Information Development, Inc., Houston

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Published 1995.
Printed in the United States of America

98 97 96 9 8 7 6 5

Library of Congress Cataloging in Publication Data

Appel, Billings, [eds.]
Oak Wilt Perspectives: The Proceedings of the National Oak Wilt Symposium.

95-75875 1995
ISBN 0-9644015-0-9

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Prelude

These proceedings are the result of presentations given at the National Oak Wilt Symposium held in Austin, Texas, in June, 1992. At the Symposium, experts in oak wilt research and management gathered from throughout the United States and reported on their experiences in dealing with the disease. From the Texas perspective, the Symposium was long overdue. Even though other states have expended considerable time and effort on oak wilt control since the disease was first discovered in 1941, losses of live oaks reached epidemic proportions throughout central Texas only during recent decades. It is only in the past 10 years that Texas has mounted a comprehensive, region-wide effort to stop the destruction of trees. Much of the disease control technology used in Texas was drawn from published reports of oak wilt management in other states. Many of those states were represented by speakers at the Symposium. At the same time, new tools were being developed and adapted to the unique conditions encountered in the central Texas live oak savannahs.

Progress in oak wilt control in Texas has been considerable, but the epidemic continues to proceed at unacceptable levels. Therefore, an *ad hoc* committee was formed to organize a meeting where all available technology on oak wilt control could be examined and discussed; the result was the National Oak Wilt Symposium. The Symposium was planned and organized with several objectives in mind. The first objective was to bring research scientists, extension specialists and agents, practicing foresters, and arborists together to discuss their successes and failures in working with oak wilt. The next objective was to present their information in such a way that interested landowners and community organizers could examine the available information and utilize the technology for disease control. Finally, the Symposium was to provide a "benchmark" in our understanding of oak wilt so that resource managers confronted with the disease in the future may have a current summary of basic and practical information on the disease.

The speakers at the National Oak Wilt Symposium were each invited because they were able to provide a different perspective on the study and control of oak wilt. They represent only a small fraction of the hundreds of individuals who have devoted efforts to better understanding and controlling the disease. Nonetheless, those present in Austin provided a valuable summary of the developments that have brought us to our current understanding of oak wilt. We are grateful to each of them for successfully fulfilling their charges.

The success of the Symposium was a reflection of the tremendous effort being expended to cope with oak wilt in Texas. Achievements have been made only because of a strong cooperative effort on the part of several different state and federal agencies and private enterprises. Each was represented or participated in the planning and organization of the Symposium. We are particularly grateful to those agencies, organizations, and businesses that donated resources to make the Symposium and this publication possible. Their names may be noted on the inside cover of these Proceedings. In addition, several individuals deserve special mention for their commitment to the oak wilt effort and the Symposium. These are: Anne Poteet-Blum, Texas A&M University; Martha Johnson, Cathy Wallace, Eugene Gehring, and Aaron Reisfield, Texas Forest Service; and Carl Schattenberg, the City of Austin. Without their dedication, this publication would not have been possible. Finally, special recognition is given to our fellow members of the organizing committee: Dr. Scott Cameron, Texas Forest Service; Dr. Jerral Johnson, Texas Agricultural Extension Service; and Ms. Susan Tracy of Medina, TX.

David N. Appel and Ronald F. Billings, Editors

Introductory Remarks

From the State Forester:

Texas Forest Service

Bruce R. Miles

It is my opportunity and responsibility to welcome all of you to Texas and to the premier oak wilt center of the United States. We have people from all over the United States attending this National Oak Wilt Symposium. I want to briefly introduce a special guest, the Speaker of the House, Gib Lewis, of the Texas Legislature. He isn't attending as Speaker of the House as much as he is a rancher with oak wilt on his own property. He has a very vested interest and if I don't convince you of anything else in the next few minutes, I hope that I can convince you that the best way to get funding for programs in oak wilt is to see that it gets on a Speaker's land or a congressman's land or somebody's land that has some influence over your budget.

We have folks here from Minnesota, Pennsylvania, Michigan, Mississippi, Wisconsin, California, and West Virginia; maybe even Arkansas. And this brings us to the natural resources problem that we are all interested in. Several years ago, as I recall, the Arkansas legislature was discussing selling water to the State of Texas. It was quite a controversy whether or not to sell water to Texas and, if so, how big a pipeline they were going to have to build. Finally, the speaker of the Arkansas legislature at that time said "All we need to do really is run a garden hose over there, and if those Texans can suck half as hard as they can blow, they won't have any trouble getting all the water they need." It's been down hill ever since with Arkansas.

As I travel around the United States and in Mexico, I have the constant problem of trying to convince people that there are forests in Texas, that there is a Texas Forest Service, and about the programs that we develop. My favorite story, which has the added advantage of being true, is one about Mr. E. 0. Siecke, the second state forester of Texas. Mr. Siecke was a state forester for 25 years. He came from the state of Oregon where he was a deputy state forester for a number of years. He set up shop in College Station where the Texas Forest Service is headquartered. His wife came some two or three weeks later by train. To get to College Station from Oregon then, one took the train out of Los Angeles through El Paso and on in to College Station. It was in El Paso that an elderly gentleman sat down next to her and they got into conversation. He said, "Well, Mrs. Siecke, just what does your husband do?" She quite proudly replied "My husband is the new state forester for the State of Texas." The man looked out the window at the barren landscape as the train headed east out of El Paso, thought a minute, and said, "I'll tell you one thing lady - they either need one damn bad or they don't need one at all." Now we have a forester in El Paso; an urban forester that works with our communities out there. We have urban foresters in Abilene, Dallas-Ft Worth, Corpus Christi, Houston, and several other areas around the state where we are working with the 80% of Texans that live on 3% of the land. But our big commercial forests are located in East Texas. We've got 11.7 million acres of commercial pine forest scattered throughout 23 million acres in East Texas. Forty three counties in East Texas employ 60 thousand people; forests support a $5.6 billion industry and are the economic engine for East Texas. For that matter, our forests make a considerable contribution to the State of Texas as well.

But I know that you are here because of the oak wilt problem in the 46 counties in central Texas, one of the most destructive plant diseases that we have in the United States. It was in the late 1970's that I was trying to get federal funds through the US Forest Service for this project. For two or three years I worked at this and then all of a sudden, I get a call from somebody in Washington, DC with the US

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Forest Service who says "We are going to give you $300,000 for the oak wilt program in Texas." When I asked how that came about, he said they had gotten a call from Congressman Pickle down in Texas. I replied, "Why is it that I have to work three years for this, and Congressman Pickle makes a five minute telephone call and I get this money. He said, "Congressman Pickle explains it better than you do."

Since 1980 we've put over $2 million in this project; we've got $1.1 million in US Forest Service money and the Texas Forest Service and private landowners have matched this amount for the project in central Texas. But it's not going to be easy to continue to do that. We had a symposium in Lufkin, Texas two weeks ago on forestry in Texas, sponsored by Congressman Charles Wilson. Congressman Wilson was quick to point out that the balanced budget amendment and other concerns were going to start drying up federal money. We already have a problem in Texas with funding our programs. Our regents have told us we are going to have to start cutting back our programs with regard to funding. So there is a considerable amount of concern for funding any program.

Despite the funding concerns, the success of this program has been phenomenal because it has involved private landowners, federal government, state agencies, and other parts of the Texas A&M University System. Let me give you a quick example. I was in Washington, DC over a year ago now walking from the capitol underground to one of the congressional buildings. Congressman Kika De la Garza came by and at the time I was talking with the former congressman from Georgia, Bill Stuckey. Bill happens to be president-elect of the Forest Farmers Association. As we were talking I excused myself to talk to Congressman De la Garza a moment. He was coming to Austin to speak at our urban forestry awards luncheon so I stepped over to visit a few minutes. Congressman De la Garza very graciously was discussing what he wanted to do out here with his speech. I went back to Stuckey and he said,"What does a congressman from south Texas know about forestry?" I said,"He's chairman of the House Agriculture Committee which has the Farm Bill which, in turn, has a little bit to do with forestry in the United States. Secondly, he and I were in Mexico City together with a congressional delegation and forestry delegation attending the World Forestry Congress. Thirdly, he's going to speak at the awards luncheon for the urban forestry program in Austin, Texas next month and he has a considerable amount of influence in forestry."

Congressman De la Garza came to Austin, and he and Congressman Pickle both were on the program. Congressman De la Garza talked about urban forestry and his involvement in the program. Congressman Pickle then received an award from us for his support and talked considerably about the amount of funding and what he could do. Also on that program was Don Wileke from the State of Minnesota. Many of you may know that Don has two problems: 1) he's from Minnesota and 2) he's a lawyer. Don is president of the National Urban Forestry Council. After the luncheon, I noticed that Don walked out of the meeting with his arm around Congressman Pickle and I knew the Texas Oak Wilt Program was in trouble. About two weeks later I got a call from the Chairman of the Forest Health Committee of the National Association of State Foresters who happens to be the state forester of Maine. He said, "Bruce, Minnesota is trying to get some of your oak wilt money." Right then things began to click back two weeks ago; I was reminded of the elderly couple in East Texas that were looking through a graveyard. The woman came upon a new grave with a tombstone that said; here lies a lawyer and an honest man. She said,"look Pa, they are putting them two to a box now."

So I just wanted to point out in my comments here the importance of this problem and that we get our legislative people involved in it. Congressman Pickle really got interested in it when oak wilt began to impact his neighborhood. Two years ago then Representative Lena Guerrero met with us in her neighborhood where we had some demonstrations on oak wilt and control measures. She made an excellent presentation on the cooperation among state and federal agencies and the private landowners and how it all worked. She is now Chairperson of the Texas Railroad Commission, a very powerful agency here in Texas, and I think we will see more from her

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in the years to come.

Representative Linebarger has oak wilt in her neighborhood and she has become very interested in it. Governor Ann Richards has signed a proclamation making this very week Oak Wilt Awareness Week, again through the efforts of Speaker Gib Lewis who talked with the governor about the importance of this program. We are very pleased that the governor is aware of this program and it is my understanding that she is in Houston with the mayors conference today and couldn't be with us. We also received a letter from Lady Bird Johnson. She is out of the country now, but also has a great interest in this disease problem, having had several oak wilt centers controlled on the LBJ Ranch.

So we appreciate the fact that we've got people from all over the United States here. In Texas I have had the unique opportunity to become acquainted with southern pine beetle, oak wilt, Africanized bees, and Ross Perot, and we welcome any relief you can offer from any or all of them. We are pleased to have you here and we hope that you will learn something. More importantly, we hope we will learn something from you.

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From the Deputy Chancellor and Dean:

T.A.M.U. College of Agriculture and Life Sciences

J. Charles Lee

I want to add my welcome to that of Director Miles. Austin is a special location for this meeting, having become known recently as the "City of the Treaty Oak." We are grateful that the tree has been given its life through the efforts of our experts, but regret the tragedy that has stricken one of Austin's most beautiful monuments. If there was any highlight from that senseless act, it was, in showing to a sometimes doubtful nation, that trees do occur west of the pineywoods region of east Texas and are held in great regard.

Twenty-five years ago, a young research forester was out on the hillside in West Virginia collecting specimens from a tree that allegedly had oak wilt. As he was thoughtfully going about his work, he heard a loud click come from behind him. As he turned around, he found himself face to face with what was the epitome of the mountaineer man with a cocked, double barrel shotgun pointing directly at him. This mountain man asked the research forester who he was and what he was doing. In reply, the forester attempted to explain the act of collecting disease specimens from a tree and why this tree was suspected to be infected by oak wilt. The man questioned the forester's sense, replying that trees die here all the time - why did he come here on his property to look at this one? The young research forester explained how he was sorry if he was on the wrong property, and after a long discussion the gun was put down. But, while I don't collect specimens from oak wilt trees anymore, I still occasionally hear that click behind me, especially in my present capacity. So I still have vivid memories of oak wilt.

I wanted to say thank you to all of those that put together a superb symposium - an excellent symposium. The intent of this symposium is to bring together scientists, tree care professionals, tree owners, and community leaders from around the country to share their experiences and their ideas with us here in Texas. Oak wilt has not been just a Texas problem in the past, but it is primarily a Texas problem at the moment. It is noteworthy, I think, that this is not a typical academic symposium where one Ph.D. talks to another, but rather involves virtually every segment of the tree industry, tree care groups, and private landowners as well.

Live oaks are a very important ecological, aesthetic, and economic resource in this state. They are expected to live for centuries, affecting both rural and urban interests. We in the Texas A&M Agricultural Program are trying to extend our expertise into the cities on behalf of this and many other serious issues of the day. All of us would agree, I believe, on the destructiveness of oak wilt, particularly in urban settings - the major, and only, silver lining I see is that the problem itself has been a significant factor in galvanizing support for urban forestry in this state.

Live oak mortality has been a problem in Texas at least since the 1930s. It has now become a focal point for the agencies that were mentioned earlier - agencies of the Texas A&M University System, the City of Austin, the USDA Forest Service, the Soil Conservation Service, and community leaders - these have all come together to form a partnership that has had a tremendous impact on what the real issues are in understanding the disease and developing control strategies. The Agricultural Experiment Station has been involved in determining the cause, testing methods of control, and understanding methods of

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spread which appear to be different in Texas than when I was a young research forester in West Virginia. The Agricultural Extension Service has had very successful, intensive educational efforts with demonstrations, media programs, and other kinds of help for landowners to understand the importance of the disease and its economic and ecological impact on their lives. The Texas Forest Service has administered a very large and very effective public assistance program across the region in addition to helping with the demonstrations for control. The City of Austin has surely been a major force in this partnership and in advocating the need for resources to state and federal sources, as has community support as evidenced by Susan Tracy and her committee in Medina. The USDA Forest Service has committed technical expertise and budget support to help us with the effort. All of these things have been very important in bringing the focus to the problem that we have here in Texas, and now bringing all of you from other parts of the country together.

We have a very effective and enthusiastic team, Speaker Lewis. I don't think we have any other situation in Texas at the moment, where community components and public agencies have come together in cooperation on such a critical issue to the state. Our challenge, it seems to me, is to assure that there is adequate resources to continue the efforts that have begun. At the federal level, it is increasingly difficult to get dollars earmarked for a particular state. The best thing that could happen for us, Bruce, I guess is for all these people to take some oak wilt home with them and spread it to other states and then it would enhance the federal role. I hope that's not what will happen, but it may take that kind of situation to garner the support necessary to solve the problem once and for all. At the present, the emphasis at the national level is being put on tree planting. This emphasis will be followed by who is responsible for nurturing all these trees that are being planted around the country today, because contrary to what people think trees normally don't care for themselves.

At the state level - Speaker Lewis can speak far better than I - about where we are in terms of resources. There is going to be intense competition for resources to meet the needs of the State of Texas in the next legislative session. I made a presentation to our Board of Regents last week who have asked all the elements in the Texas A&M University System to take a look at efficiency, effectiveness and priorities, and make decisions as to what we would do with less funds than we have available today. There is an expectation at both the federal and at the state levels that the beneficiaries of programs will provide an increasing share of the resources that are needed to meet urgent needs. The thing that helps us the most in our conversations at the state and federal level is to be able to show, first of all, that we have effective programs. Secondly, we must demonstrate that we are leveraging the resources that are entrusted to us by the state with private resources or federal resources. And, thirdly, we are working together as a community, just as the TAMU agencies and cities such as Austin, along with the private sector, are doing to make the oak wilt program a successful effort. Those are the kinds of things that give us confidence in speaking with our Board and with people like Speaker Lewis who have a tremendous responsibility in the allocation of the state's resources. Those are the kinds of partnerships that are absolutely essential if we are going to have the resources to confront problems such as oak wilt.

I am delighted to see so many people here including some former students of mine from Texas A&M and other parts of the country. I wish that I could stay with you for the duration of this session, but I have a meeting with representatives of the tourist industry to explain to them what we are going to do to help the horse racing industry make enough money for the State of Texas so that we can afford to solve this oak wilt problem. I want to say special thanks to Speaker Lewis for all that he does for the agricultural programs of the Texas A&M System - we appreciate that support very much. We're sorry that you have to put up with the pest, but it will give these people some more stimulation to solve the problem. Thank you very much, I appreciate the opportunity to be here.

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Section I
Current Understanding of Oak Wilt

Oak Wilt: An Historical Perspective

William L. MacDonald

Few forest pathogens are as capable of killing their hosts as is *Ceratocystis fagacearum*, the incitant of oak wilt. This organism causes a vascular disease of oaks, chestnuts, chinkapins, and tan oaks. The most susceptible species are members of the red oak group. White oaks are tolerant of the disease but no oak species is known to possess immunity. Following transmission by insects or root grafts, the fungus invades the vascular tissues and causes host responses that result in dysfunction of the xylem transport system. Susceptible trees express wilting symptoms soon after infection and most die within a year. Oak wilt is known only in the United States and until recently was associated with the oak forests of the central and eastern United States. Even though the disease can be important locally it has not caused wide-spread economic losses to our highly valued oak forests in these regions. The disease is considered internationally dangerous because its potential on other continents is unknown. The current experience with oak wilt in Texas demonstrates the destructive potential of this pathogen.

INTRODUCTION

Oak wilt was first described as a disease fatal to oaks in a 1942 Wisconsin Agricultural Experiment Station Report (Anonymous 1942). Two years later a previously unknown fungus was reported as the inciting agent and was named *Chalara quercina* based on the asexual stage produced in culture (Henry et al. 1944). Recognition and proof that the fungus occurred in other states came rapidly. By 1951, the disease had been recorded in 18 states from Minnesota to Pennsylvania in the North and from Arkansas to North Carolina in the South (Fowler 1952). Successive reports of discovery in most cases were the result of recognition rather than of disease spread. Its current distribution includes a few additional southern states with the most destructive outbreaks in Texas. Some evidence suggests that the disease was causing death of oaks at the beginning of the century in Wisconsin and Minnesota (Gibbs and French 1980). Various theories as to the origin of *Ceratocystis fagacearum* (Bretz) Hunt are reviewed by True et al. (1960). Most investigators have discounted the possibility that *C. fagacearum* is of foreign origin because it is difficult to envision a fungus becoming established over such a wide area when local dissemination occurs so poorly. An effort to predict the course of the oak wilt epidemic in Pennsylvania and West Virginia was made by Merrill (1967). Factors influencing the distribution of *C. fagacearum* are complex and poorly understood.

DISEASE SYMPTOMS

The external foliar symptoms of oak wilt usually include water soaking and browning or bronzing of leaf tips and margins. Leaf abscission is common to both green and symptomatic leaves and may result from the formation of an abscission layer that is anatomically similar to that normally produced in the fall (TeBeest et al. 1973). For most members of the red oak group (subgenus Erythrobalanus), nearly the entire crown shows symptoms soon after the disease is evident. Short-lived suckers may develop from the bole and larger branches of defoliated trees either in the same year or in the year following defoliation.

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The course of symptom development in white oaks (subgenus Lepidobalanus) is less predictable. Trees may either display foliar symptoms branch by branch, year after year, until the entire crown dies, or recovery can occur, especially in larger trees. Veinal necrosis is most diagnostic for live oak but this symptom is not always observed (Appel 1986).

THE CAUSAL FUNGUS

Henry (1944) described *Chalara quercina* as having white mycelium, becoming gray to olive green with occasional patches of tan. The septate hyphae produce branches subhyaline to brown with conidiophores that are not sharply differentiated from sterile hyphae, except for some taper near the apex. The endogenously produced conidia are hyaline, cylindrical, 1-celled, truncated at both ends, and measure 1-4.5 x 4-22µ.

The sexual stage of *C. quercina* was discovered by Bretz (1952), who renamed the fungus *Endoconidiophora fagacearum* Bretz. He discarded the species epithet as the fungus was known to be pathogenic to other genera within the Fagaceae (Bretz 1955, 1957). In 1956, the fungus was reclassified to the genus *Ceratocystis* in a monograph by Hunt (1956). The black perithecia are flask-shaped with spheroidal bases and have necks 250-450µ long that terminate with a fringe of hyaline hyphae. Asci are globose to subglobose, evanescent, and contain eight hyaline, one-celled ascospores that are elliptical and slightly curved. Ascospores measure 2-3 x 5-10µ and accumulate at the ostiole in a sticky, creamy-white mass. Details of the morphology and cytology of perithecia and ascospore development are provided by Wilson (1956).

Bretz (1952) concluded that the fungus was heterothallic as perithecia did not form in nonpaired single-conidium and single-ascospore cultures. The nature of the heterothallic condition was explained when Hepting et al. (1952) demonstrated that conidia could act as spermatia when transferred to a thallus of the opposite type. The thallus acts as the female, and perithecia are usually produced within three to four dys. after spermatization. Each single-spore culture was shown to be bisexual and a member of one of two mating groups, arbitrarily designated A or B. Both mating types have been found throughout the range of oak wilt and in most small local areas in a frequency of about 1:1 (Appel et al. 1985, Barnett 1952, Kaufman and MacDonald 1973).

Ceratocystis fagacearum produces a unique fruity smelling mat between the bark and the wood on some infected trees (Struckmeyer et al. 1958). With the proper environmental conditions the mycelium of the fungus accumulates at certain points in the cambial region to form the sporulating mats. They are a tightly woven mass of mycelium that may extend over several square inches and produce abundant conidiophores and endoconidia (True et al. 1960). Near the center of the mat a pair of thick, round, or elongated, cushion-like structures may develop. The cushions are formed back to back, one attached to the bark and the other to the wood, and create enough pressure to raise and often rupture the bark. Internally, the cushions are divided into a daedloidlike pattern with inflated chambers. The term "pressure cushion" has been used to describe these structures which may serve an important role in survival and dissemination of *C. fagacearum*. Pressure cushion formation is most common to the susceptible red oaks including Spanish and Texas oak and rare or absent in white oaks or semi-evergreen live oaks (Appel 1986).

THE DISEASE CYCLE

Survival and Dissemination of *Ceratocystis fagacearum*

The survival of *Ceratocystis fagacearum* depends on its association with host material. After killing a tree, *C. fagacearum* competes poorly with other fungi that colonize dead oaks, indicating a poor adaptation to a saprophytic existence apart from recently killed trees (Shigo 1958). Survival therefore is related intimately to continued dissemination and reestablishment of the host-parasite relationship. *Ceratocystis fagacearum* survives little more than 1 yr in the boles of infected trees (Merek and Fergus 1954) and occasionally as long as 4 yr in root systems (Amos and True 1967, Yount 1955). However, in most roots, survival is short, probably due to antagonism or competition by other organisms (Amos and True 1967). The fungus

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is not freeliving in the soil (Berry and Bretz 1963). *Ceratocystis fagacearum* also can survive in dead trees, felled logs, and even infected lumber (Englerth et al. 1956, Tainter et al. 1984). Some infected leaves may harbor *C. fagacearum* for a few months, but it is unlikely they are a source of inoculum (Hager 1962). The results of numerous survival studies have been variable with authors often giving temperature, moisture, and competing organisms as factors limiting survival (True et al. 1960). When wood moisture was controlled, isolation from sapwood decreased with lower moisture levels, until at 20% moisture no successful isolations were made (Partridge 1961).

Ascospores generally survive longer than conidia (Merek and Fergus 1954). Survival of both is better with cool temperatures and low relative humidity. A detailed account of spore survival is given by Curl (1955). Studies of conidia and ascospores associated with insects proved that the fungus could survive for extended periods on dead or dormant nitidulid beetles (Stambaugh and Fergus 1956) and that conidia remained viable after passage through the bodies of the beetles (Jewell 1954).

Ceratocystis fagacearum may spread from root systems of diseased trees to healthy trees by root grafts (Kuntz and Riker 1950). The rapid spread of the organism to adjacent trees commonly creates large centers of infection in the Lake States region (Anderson and Anderson 1963). In the Appalachians, the occurrence of large spreading oak wilt centers is less frequent, yet tree-to-tree infections by root grafts cannot be discounted as an important means of dissemination and pathogen survival (Boyce 1957, Staley and True 1952). In Texas, the rapid rate of oak wilt disease center expansion in live oak is attributed to the high potential for root grafting and the occurrence of common root systems among clonally propagated live oaks (Appel et al. 1989).

Insect vectors may be responsible for local and long-distance dissemination of *C. fagacearum* (see Merrill and French, these proceedings). The first nsects associated with transmission of the oak wilt fungus were the nitidulid beetles (Dorsey et al. 1953, Norris 1953), which apparently are attracted to the fruity aroma of mats produced between the bark and the wood on some infected trees. The insects frequently visit the fragrant mats (Boyce 1954) and acquire inoculum necessary to transmit the fungus (Dorsey and Leach 1956). Being sap feeders they could potentially transmit spores to fresh sap wounds on healthy trees. They also can spermatize mats by transmitting conidia among mats of different mating types (Leach et al. 1952). When this occurs, perithecia develop and produce ascospores, providing additional inoculum. Proof that nitidulids could serve as vectors came from experiments where spore-carrying insects were caged in freshly-wounded healthy oaks (Dorsey et al. 1953). A review of the suspected role of oak bark beetles (*Pseudopityophthorus* spp.) and other insect vectors is given by Rexrode (1976). Even though insects apparently are the principal vectors for long-distance dissemination, many questions still remain as to the relative importance of specific insects in different oak wilt regions.

Infection, Colonization, and Host Response

Infection by *C. fagacearum* occurs either by movement of the pathogen from tree to tree through root grafts and common root systems or by growth of inoculum introduced into wounds by vectors. Even though trees that have been artificially inoculated throughout the year produce symptoms, most natural infections occur from mid-May to early July, when insect activity and available inoculum presumably are greatest (Jeffrey 1953, Norris 1955). Movement of *C. fagacearum* through root grafts has not been associated with season. Peak mat production and nitidulid activity in Texas occurs in late winter (Appel et al. 1987).

Once introduced into a susceptible oak, the fungus spreads rapidly within the current year's xylem vessels by either hyphal growth or the production of numerous conidia carried by the transpiration stream (Jacobi and MacDonald 1980, Young 1949). At the time of symptom expression in red oaks, colonization is complete and the fungus can be isolated from roots, stems, branches, twigs, petioles, and leaf midribs (Henry and Riker 1947, Young 1949). The fungus begins to colonize inner growth rings when the tree dies (Young 1949), and has been isolated as far into the sapwood as the tenth annual ring (Jones

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and Bretz 1955). Heartwood is not colonized because it contains substances toxic to the fungus (Bilbruck 1959).

Young (1949) suggested that the conidium is the major propagule for movement of the fungus within host tissues, because he was able to force suspensions of conidia through 12-in sections of red, white, and bur oaks. Yet, conidia rarely have been observed in xylem vessels, and no fungal propagules were found in pressure washings taken from infected stem sections (Gregory 1971, Parmeter et al. 1956, Struckmeyer et al. 1958, Wilson 1961). Observations of conidiophores are even more uncommon (Jacobi and MacDonald 1980, Wilson 1961).

Lateral movement of the fungus is accomplished by hyphal penetration of pits and cell walls (Jacobi and MacDonald 1980, Nair 1964, Wilson 1961). Inter- and intracellular growth (Sachs et al. 1970) and invasion of parenchyma cells, especially after symptom expression, also have been observed (Struckmeyer et al. 1958). The amount of mycelium observed in vessels and tracheids prior to wilting is limited (Parmeter et al. 1956, Wilson 1961, Young 1949).

The distribution of the fungus in resistant white oaks is more restricted than in susceptible red oaks. The normal lack of vessel interconnections in white oak may confine the pathogen to narrow vertical sections of the bole and to branches with vascular connections (Nair 1964, Parmeter et al. 1956). These conclusions were supported by dye studies in which serial cross sections of the boles were examined from the point of dye introduction; the width of the dye arc increased in susceptible oaks but decreased in resistant ones (Parmeter et al. 1956).

Ceratocystis fagacearum apparently incites responses within the host that cause occlusion of the vascular elements of the xylem. The formation of tyloses is the first anatomical response associated with wilting in red oaks (Cobb 1963, Jacobi and MacDonald 1980, Nair 1964, Struckmeyer et al. 1958). At the first sign of wilting, tyloses have been reported to occlude 100% of the current year's vessels in twigs and 50-70% of the vessels in single trunk cross sections (Beckman et al. 1953, Struckmeyer et al. 1954). Plugging of vessels was observed two to five dys. before permanent wilt (Parmeter et al. 1956) when drastic decreases in water movement also occurred (Beckman et al. 1953). Gregory (1971) found resistance to water flow in stems of 1 to 2-yr-old red oak seedlings eight dys. after inoculation. In addition, he was able to correlate increased resistance to water flow in stem and petiole sections with the first appearance of the fungus in that particular stem section. TeBeest et al. (1976) showed that stomatal resistance in red oaks increased sharply approximately 3 dy before visible symptoms. Final proof that oak wilt symptoms are caused by vascular occlusion may result when the water relations of infected oaks are examined.

Gummosis of small vessels and tracheids has been observed during late stages of wilt (Struckmeyer et al. 1954). Nair (1964) also described parenchyma cells filled with darkly stained granular material and fat-like droplets. Jacobi and MacDonald (1980) confirmed Nair's observations and further described bubble-like structures within vessels colonized by *C. fagacearum*. High molecular weight polysaccharides are produced when *C. fagacearum* is grown *in vitro* (McWain and Gregory 1972). These compounds can cause necrosis and wilting when injected into seedlings and may account for the bronzing of leaves in diseased oaks. Indole acetic acid (Fenn et al. 1978) and pectolytic enzymes (Fergus and Wharton 1957) also are produced *in vitro*. The precise role these compounds play in disease development must still be addressed.

The mechanisms responsible for resistance in white oaks are largely unexplained. Differences in host anatomy and/or responses to infection have been observed that may enable a resistant host to more effectively localize the fungus within infected xylem elements (Jacobi and MacDonald 1980). In some resistant oaks an atypical band of xylem, composed of large open vessels, forms external to infected early wood vessels (Schoenweiss 1959). Several theories for this occurrence have been mentioned (Cobb 1963, Marchetti 1962) and the possible role of indole acetic acid in cambial reaction time has been investigated (Lacasse 1966a,b). Yet, neither the frequency of this phenomenon nor its relationship to resistance is known.

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FACTORS INFLUENCING THE COURSE OF DISEASE

Host Influences

In most regions where *C. fagacearum* occurs, susceptible host material is abundant. Yet, host abundance and disease incidence evidently are related only in areas where stem density results in frequent root grafting. This is especially the case in the north central states and in Texas where an underground network of root grafts or common root systems results in the development of large infection centers (Appel et al. 1989, Gibbs and French 1980). The relationship between root grafting and disease incidence in the Appalachians may be more important than previously thought, since evidence suggests that several years can elapse between the death of one tree and root graft transmission to another (Rexrode 1978). Thus, many infections previously attributed to vector spread may actually be the result of delayed root graft transmission.

The movement of the pathogen through root grafts between resistant and susceptible oaks occurring in mixed stands has not been shown (Jones and Partridge 1961). Infection of resistant species then must be from vector inoculation or intraspecific root grafts. Even though resistant trees and related species, like American chestnut, may harbor the fungus for several years, they probably contribute little to the reservoir of inoculum (Merrill 1975). The possibility that resistance exists in the red oak population is supported by the finding that a few red oak seedlings survive repeated inoculations (Pengelly et al. 1977). However, the frequency of resistant red oaks among populations of older trees is unknown.

Influences of Pathogen Variability

Cultural variability among isolates of *C. fagacearum* is well known, but there have been few attempts to deal with the importance of pathogenic variability. Barnett (1952) failed to find evidence for geographic races among 100 isolates from nine states. Likewise, no evidence for host specificity was found when cross inoculations were made with isolates from wilting trees of the white and red oak groups (Henry and Riker 1947, Young 1949). Differences in mat production and incidence of infection were noted when seven isolates from different states were inoculated into red and chestnut oaks (Cobb and Fergus 1964). In this Pennsylvania study, typical mats were produced by isolates from Arkansas, Missouri, and Ohio; thus other factors, most probably climatic, must be responsible for the rare occurrence of mats in those states. Because few replicates were used and the cultures were of different ages, the authors warned against drawing conclusions about differences in pathogenicity.

A highly replicated study using 30-40 day-old red oak seedlings was designed to test the pathogenicity of 24 isolates from the northcentral, southcentral, and Appalachian oak wilt regions (Haynes 1976). Significant differences in pathogenicity occurred among isolates, but no region yielded isolates significantly more virulent than another. Haynes (1976) warned that differences in isolate pathogenicities as detected by the seedling test may or may not be significant in larger trees. Furthermore, in conducting such tests, the isolates obviously were selected because they kill trees. Whether weakly pathogenic strains that do not produce symptoms occur in the population is unknown.

Vector Influences

Overland dissemination of *C. fagacearum* is considered to occur by animal vector. With the production of sticky spores and mats, *C. fagacearum*, like many other *Ceratocystis* species, seems well adapted to insect dissemination (True et al. 1960). Yet, the nitidulid and oak bark beetles that commonly have been associated with overland transmission must be very inefficient, or the incidence of oak wilt would be considerably greater. Successful transmission by nitidulids requires that they first acquire inoculum from mats and then visit fresh wounds to introduce the fungus (Juzwik and French 1983). However, with the exception of Texas (Appel et al. 1985), mats rarely form in the southwestern portion of the oak wilt range (Berry and Bretz 1966) or in other regions during drought years (Boyce 1957). Because the presence, absence, or frequency of mat production have not been related to the rate of disease spread

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(Himelick and Fox 1961), fresh sap wounds may be the factor limiting transmission by these beetles in areas where mats exist.

For oak bark beetles to transmit the fungus they must either emerge from breeding galleries carrying inoculum or must acquire and transmit the fungus during their feeding activities (Rexrode 1968). Transmission from brood trees that died the preceding season is unlikely as the fungus seldom survives over winter in the small branches where breeding occurs (Bretz and Morison 1953, Peplinski and Merrill 1974). It also is doubtful that beetles which emerge during the summer and feed among infected and healthy oaks are vectors because most inoculations of oak appear to occur from late May to early July (Jeffrey 1953). Finally, the experimental evidence for transmission by any vector is weak, further emphasizing that the prerequisites for vector transmissions are seldom met.

Environmental Influences

Convincing relationships between environmental or site factors and disease incidence have been difficult to establish. In early Wisconsin studies, the severity or rate of disease spread could not be correlated with soil type, available nutrients, site, age, or size of trees (Henry et al. 1944, Kuntz and Riker 1950). However, Anderson and Anderson (1963) indicated that the prevalence of infection and damage in Wisconsin and Minnesota appeared to be greatest among scrub oaks that grew on poor sites. In West Virginia, Gillespie and True (1959) found that shallow soil, a factor usually associated with poor site productivity, favored local spread in five northeastern counties. Oak wilt centers were found to be more prevalent on ridges and upper slopes in Pennsylvania and West Virginia (Cones 1968, Jeffrey 1953). Cones (1968), however, suggested that the association of oak wilt with the poorer sites on ridges and upper slopes related more to the environmental factors affecting the host, pathogen, or vectors on those sites than to quality of the site. Rexrode et al. (1965) reported that site and aspect did not influence the incidence of the oak bark beetle.

The role of high temperature and *Hypoxylon atropunctatum* (Schw. ex Fr.) Cke. in limiting the southern distribution of the pathogen is discussed by Tainter and Gubler (1973); it is one of the more convincing relationships between environment, a competing fungus, and disease incidence. The relationship between high temperature and *H. atropunctatum* apparently does not exist in areas of Texas where oak wilt is prevalent (Appel et al. 1985). The interaction of *C. fagacearum* with numerous other fungi from diseased oaks has been considered (Shigo 1958). Some of these fungi are capable of checking or killing *C. fagacearum in vitro* and also may influence the survival and production of inoculum by *C. fagacearum* in the forest.

Human Influences

The role of human activity on the distribution of *C. fagacearum* is difficult to assess. However, it is documented in some regions that local spread, presumably by nitudulid beetles, occurs when tree wounds are made in the spring (Craighead and Nelson 1960, Juzwik and French 1983). This has become evident to home owners and contractors who have pruned or damaged trees around home sites within stands of red oak (French and Stienstra 1975). The overland movement of *C. fagacearum* infected logs could account for the occasional reports of new oak wilt infections found outside the known range of the disease, but no documentation for this exists (Grand and Doggett 1973, Witcher 1969).

Perhaps man's greatest influence on the disease resulted from extensive logging that was common to most areas where *C. fagacearum* now exists. In many cases, the logging resulted in changes of species and the natural diversity of the forest. For instance, in central Wisconsin, oak wilt has been severe in coppice stands of northern pin oak (*Quercus palustris* Muenchh.) that became established after logging and fire destroyed much of the original diverse woodland (Gibbs and French 1980). Any changes to the forest that alter host populations may in turn modify relationships of vectors, root grafts, and environment that could in turn influence disease incidence.

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Control

Numerous control procedures have been employed over the years to reduce the incidence of oak wilt. Most researchers agreed that interrupting the movement of *C. fagacearum* through root grafts or eliminating transmission by vectors were two practical ways to break the disease cycle. Therefore, numerous procedures to chemically or mechanically disrupt root grafts were tested and found to be most suitable to the deeper soils of the North Central States (True et al. 1960). In Pennsylvania, a barrier to root graft transmission was created by felling all healthy potential hosts within 50 ft of diseased oak trees and poisoning the stumps (Jones 1965). Most recently, Bruhn et al. (1991) have provided a model that can be used as a guide to root graft barrier placement in the Lake States.

With the discovery of sporulating mats on infected trees, various methods to prevent their formation were tried. In West Virginia, the technique of "deep girdling" diseased trees to the heartwood to hasten drying and thus reduce mat production was used for many years as a control measure (True et al. 1960). Chemicals and biological control agents also have been pressure injected into diseased oaks and evaluated for their effectiveness in reducing mat formation, beetle breeding, and root graft transmission (MacDonald and Double 1978, Rexrode 1977). The fungicide propiconazole has shown promise as a preventative and presymptomatic injection treatment for oak wilt in live oak (Appel and Kurdyla 1992).

Evaluation of control measures has been made difficult by the slow and sporadic nature of disease spread in many areas. When an appraisal of oak wilt control programs in Pennsylvania and West Virginia compared disease incidence in control and check areas, the findings were discouraging (Jones 1971). The Pennsylvania barrier-control method was effective, but an unacceptably high number of healthy trees were destroyed. The West Virginia "deep girdle" control procedure was less expensive to apply and did not destroy healthy trees but gave inconsistent results. The questionable value and high cost of various survey and control programs, and the continued low incidence of oak wilt in many regions, have resulted in the elimination of active control programs for oak wilt in most states. The magnitude of the oak wilt problem in Texas serves as a reminder that this is a most destructive pathogen and one that is not readily controlled.

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Epidemiology of Oak Wilt Outside Texas

A.J. Prey
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Although local oak wilt epidemics have killed thousands of oak trees in central Wisconsin and southeastern Minnesota, large scale epidemics in eastern states have not developed. Differences in stand history, composition, soil, management, and human activities as well as in climate and weather conditions probably account for differences in disease impact. Meanwhile, epidemiological evidence has enabled the formulation of effective control measures which combine both preventive and curative measures.

INTRODUCTION

In the late 1800s and the early 1900s, dying oaks in the upper Mississippi River Valley were reported by farmers, woodsmen, agriculture officials, and village residents. Based on observations, impressions and circumstantial evidence, various reasons were suggested, including *Armillaria* root rot, two-lined chestnut borer, bark beetles, drought, and even "the prairie reclaiming its own."

Soon, in the 1930s, representatives of universities, state and federal agencies, forest and wood-using industries appraised oak losses, expressed their serious concern over the potential threat to eastern hardwood forests, and urgently recommended studies and research to map disease occurrence, detail symptomatology, identify causal agents, clarify epidemiology, and formulate possible control measures as soon as possible. Studies began in central Wisconsin in the late 1930s (Henry 1944, Henry et al. 1944).

Survey

From the early 1900s to 1950, oak wilt in Wisconsin was confined to the southwest and central areas. For 50 yr, the disease remained south of an ecological tension zone across Wisconsin (Curtis 1959). This tension zone divided Wisconsin into two distinct vegetation provinces; the prairie-forest province in the southwestern half and the conifer-hardwood province in the northeastern half of the state. The major plant communities of the southwestern province are southern hardwood forest, oak savanna, and prairies, while those of the northeastern province are conifer-hardwood forest, pine savanna, and boreal forest. The tension zone is correlated with several climatic factors including annual summer temperature and precipitation. Then, with increased development of vacation homes, tourism, and new recreational activities, the disease appeared in widely scattered oaks just north of this zone. New oak wilt pockets were initiated. Apparently, wounding of oak trees by human activities, when the trees were susceptible to infection, led to new local epidemics.

A similar situation occurred in the upper peninsula of Michigan where oak wilt contaminated firewood was introduced into a recreation community (Bruhn et al. 1988). Also, new subdivisions in the Minneapolis-St. Paul, Minnesota area have created similar local epidemics. However, previously reported pockets of oak wilt 100 mi north of the Twin Cities are no longer active (T. Eiber, personal communication).

Initial ground surveys and later aerial surveys found the sudden dying of oak in southern Wisconsin, but absent in northern Wisconsin. After a few years, active wilt pockets were encountered in Wisconsin, southeastern Minnesota, and eastern Iowa. Soon, dying oaks were found in adjacent states of the Midwest. Pockets of dead and dying oaks were especially numerous and destructive in central

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Wisconsin, but absent in northern Wisconsin. After a few years, active wilt pockets involved up to several acres and contained dead and decaying skeletons in the center, ringed by recently-killed trees with loosening bark -- some with fungus mats, and dying trees in various stages of wilt along the periphery. Rarely did red and black oaks escape this outward invasion and if bypassed, they usually became infected within a few years. Basal sprouts seldom arose from standing wilt-killed trees, but occasionally from stumps of cut dying trees. These sprouts, in turn, soon wilted and died. In contrast, adjacent bur (*Q. macrocarpa* Michx.) and white oaks escaped such tree-to-tree spread and were the only oaks remaining alive in a wilt area (Kuntz and Riker 1956). In urban areas where individual oaks are prized for their many aesthetic values, oak wilt was of equal or even greater concern (French 1991).

Meanwhile, national surveys were organized and have reported dying oaks in certain eastern states. The disease is widespread in West Virginia, western Maryland, and in Pennsylvania west of the Appalachian Mountains, but has not been reported from other northeastern states containing oak timber types (USDA Forest Service 1991). Spread has been limited and losses are minor. Later, oak wilt was detected and reported in some southern states, but its role and possible impact are yet unknown.

Basic Information

Early research identified the incitant of a new, highly destructive oak disease, determined its hosts and their different disease reactions, discovered its means of spread, and suggested certain control measures (French 1991, Kuntz and Riker 1956). Many aspects of its epidemiology already have been clarified (Beckman et al. 1953, Drake 1956, Houston 1961, Parmeter 1955).

The fungus spreads among its oak hosts in at least two different ways. In one case, it "jumps" considerable distances -- anywhere from a few hundred yards to more than a mile, infecting scattered previously healthy oaks. Several insect vectors have been implicated. A second type of spread is the progressive, continuous or intermittent movement in local areas from diseased to adjacent healthy trees through root grafts (Kuntz and Riker 1950).

EPIDEMIOLOGICAL FACTORS

Epidemiology considers the relationships and associations of the various factors influencing the initiation, development, and spread of a disease. Manipulation of such factors may provide control measures. Hopefully, this will be possible with oak wilt. With the oak wilt disease, several critical factors determine the incidence, time, location, severity, and impact of disease epidemics.

Pathogen

The fungus pathogen, *Ceratocystis fagacearum* (Bretz) Hunt, proved to be virulent, aggressive, lethal, and systemic. Being a vascular pathogen, the fungus lives and functions more within the host than outside the host. In turn, initial disease reactions and symptoms develop within the host but later incite visible symptoms outside the host.

Hosts

Its many hosts range from highly susceptible to somewhat resistant, but all *Quercus* and closely related species in the family Fagaceae have proven susceptible to natural or artificial inoculations. In addition, the causal fungus can infect certain related trees. Six susceptible species closely related to oak are: Chinese chestnut, *Castanea mollissima* Bl.; American chestnut, *C. dentata* Borkh.; Spanish chestnut, *C. sativa* Mill.; Allegheny chinquapin, *C. pumila* Mill.; tanbark-oak, *Lithocarpus densiflorus* Rehd.; and bush chinquapin, *Castanopsis sempervirens* Dudl. (Himelick and Fox 1961).

Environment

Climatic and changing seasonal environmental conditions determine the incidence, development, and severity of most diseases, including oak wilt. With oak wilt, even daily weather changes influence disease incidence and development (Nair 1964).

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Means of Spread

For epidemics to develop, a pathogen must have an effective and efficient means of dispersal. Although spores of the oak wilt fungus appear not to be windborne, they are dispersed in two very different ways. One way is by insect vectors, especially sap and fungus feeders (Nitidulidae). These insects are attracted not only to the fungus mats on recently killed trees but also to fresh wounds on otherwise healthy oaks. Nitidulid beetles may carry the fungus spores over long distances and initiate new infections. Unfortunately, humans also may carry the fungus in infected wood while human activities among oaks create many wound potential infection courts. Also, natural, underground root grafts between adjacent oaks in local areas enable further tree-to-tree spread of the fungus pathogen. With time, more and more trees become infected.

LOCAL AREAS

Early detection and appraisal surveys in Wisconsin and other north central states judged oak wilt to be "the greatest potential threat" to the vast hardwood forests of eastern United States, especially to the extremely valuable oak timber. In Wisconsin, thousands of oak wilt "pockets" of various sizes already exist. Local epidemics have killed and continue to kill countless oaks each year. The annual reduction, both in timber harvest and value, is substantial. Considerable loss in aesthetic values occurs with the dying of shade and ornamental oaks along city streets, around homes, and in parks and recreation areas. Especially in central Wisconsin, several prevailing conditions contribute to local oak wilt epidemics.

Stand Composition and History

Relatively pure stands of even aged, highly susceptible black oak species extend through several counties. Many oak areas have been cut or burned frequently and have regenerated vegetatively in dense stands of stump and root sprouts. Northern pin oak, *Quercus ellipsoidalis* E.J. Hill, is a major species and is very susceptible to oak wilt.

Regeneration and Root Grafts

As a result of repeated cutting and vegetative regeneration, natural underground root grafts are abundant and widespread. Intraspecific root grafts are common within the red oak group, but are much less frequent among trees of the white oak groupp. Interspecific root grafts are very rare. Thus, the underground connections furnish the fungus direct "pipelines" from an infected oak to one or more adjacent healthy oaks, especially among red or black oaks. In one experiment, 28 roots of two northern pin oaks (6 in. and 10 in. in depth, respectively) were found to be grafted (Riker and Kuntz 1956). Such root connections and their function may be prevented or broken by physical barriers such as pipelines, trenches, roads, foundations and basements, ditches, or streams.

Stand Conversion

Following the passage of oak wilt through mixed oak stands, red and black oaks are killed, leaving white and bur oaks as "escapes." Not only are the latter species resistant to disease development, but also possess few, if any, root grafts -- especially interspecific grafts. Thus, the composition of infected oak stands can change substantially. Nevertheless, cases of tree-to-tree spread among bur or among white oaks have been reported. Such spread may be slow and erratic.

Soil Type

The light, dry, infertile sandy soils of central Wisconsin appear to favor the development of extensive networks of lateral roots in the upper soil layers, mostly within 1 m of the soil surface. The movement of dyes, poisons, and radioactive isotopes, as well as fungus spores, demonstrated that one or more (often several) oaks commonly are connected by several functional root grafts (Beckman and Kuntz 1951).

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Inoculum

Existing, active oak wilt pockets with currently dying oaks along their periphery provide abundant and continuous natural inoculum, both internally for continued local tree-to-tree spread and externally for spread over long distances by insect vectors. At the same time, infected but surviving bur and white oaks also bear sporulating fungus mats and provide inoculum for insect vectors.

Meanwhile, humans -- especially those with both summer and winter homes -- may transfer fungus and insect-infested oak fuel wood from a diseased to a healthy oak locality. Conditions favoring mat production and sporulation, vector transmission, host susceptibility and fresh wounds, infection and disease development together may lead to new infections and an initiation of another oak wilt pocket. Oak loggers may act in a manner similar to homeowners. Such action by loggers, buyers, and shippers of infested oak logs has state, regional, national, and even international implications.

Infection Courts

In Wisconsin and other Lake States, fresh wounds of different kinds inflicted on oaks by many agents may provide receptive infection courts for spores carried by different contaminated insect vectors. Unfortunately, human activities -- especially during seasons and periods of high susceptibility in oaks -- create sensitive wound infection courts. Widely scattered, single-tree infections may lead to many subsequent oak wilt pockets. In many instances, citizens have paid premium prices for prime building sites in wooded areas -- only to have their prize oaks killed one by one because of their own ignorance, carelessness, or unconcern.

All too often, various public utilities (electric, telephone, water, sewer, gas, or street) have made similar blunders with the same dire consequences. Increased tourism and outdoor recreational activities in Wisconsin's parks and forests pose additional risks of tree wounding and subsequent infection. Thus, new oak wilt infections in previously-wounded trees confirm the importance of fresh wounds.

Fortunately, an increasing number of citizens have become aware of and knowledgeable about oak wilt, its spread, and possible control. Especially relevant are builders and resident associations; public utilities; private city, state and federal foresters; commercial arborists; park managers; woodland owners; and others. With oak wilt, concerned citizens have become believers and practitioners of "preventive medicine" in avoiding, preventing, or treating wounds. To their sorrow, others have learned that "curative medicine" -- once the disease is well established in active pockets -- is difficult, costly, and perennial.

Vectors

Although several possible insect vectors have been identified, certain sap and fungus feeders -- especially nitidulid beetles -- appear to be major long distance carriers of the oak wilt fungus in Wisconsin and other north central states. Sporulating fungus mats provide abundant inoculum and fresh wounds on healthy oak trees serve as opportune infection courts. The common picnic beetle, for instance, overwinters as an adult or larva in decaying organic matter, and becomes active during warm periods in early spring, commonly in April at least a month before oaks become physiologically susceptible. The vectors are present throughout the growing season, including early spring and late fall.

Humans also can be indirect carriers of the oak wilt fungus as they transport infected oak logs and fuel wood into disease-free areas. Wefts of oak wilt fungus mycelium first formed on exposed cut ends of infected oak logs stored in a warm (20-24°C), moist (100% RH) greenhouse; later, mycelium developed under loosening bark and in insect galleries.

Environmental Conditions

The oak wilt fungus survives both summer and winter climatic conditions in north central and eastern United States. Summer conditions of temperature and moisture generally favor its growth, sporulation, and spread. The fungus survives winter conditions within infected oaks and can be isolated from stems and roots of infected ones throughout the year. Even

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when air temperatures become extremely high in midsummer, so that attempts to isolate the fungus from twigs and branches of infected trees become erratic, the fungus survives in root systems and recolonizes above-ground parts when favorable conditions return.

But seasonal, periodic, and even daily weather conditions influence infection processes, incubation periods, rate and severity of disease development, mat formation, and subsequent spread (Drake 1956, Houston 1961, Kozlowski et al. 1962, Nair 1964). Critical factors include air and soil temperatures, soil moisture, and even periods of high rainfall -- especially during hot, dry summers (Drake et al. 1956, McMullen et al. 1960, Nair 1964). Air temperatures of 32°C or above inhibit or even kill the fungus in culture, in young infected seedlings, in twigs and small branches, and in logs.

Host Physiology

Both red and white oaks are most susceptible to overland, vector transmission in the spring and early summer, a period of high physiological activity and rapid growth (Drake 1956, Juzwik et al. 1985, Nair 1964). At this time, cambium actively forms large, open springwood vessels which, if severed, aspirate fresh wounds and permit the rapid and extensive movement of spores in the transpiration stream. In Wisconsin, this period of high susceptibility usually is early May through late June or from bud swell to full leaf expansion. This period of high susceptibility varies geographically with oak phenology. Susceptibility of wounds to both natural and artificial inoculation generally is much reduced in midsummer, fall, and winter.

REGIONAL AREAS

Oak wilt remains a serious potential threat to our vast and extremely valuable oak stands in north central and eastern United States. Fortunately, in most areas, annual spread has been limited and losses in oak timber relatively small. So far national epidemics have not occurred. Suffice it to say that the pessimistic predictions of early investigation have not occurred. Nevertheless, recently the threat of oak wilt to oak woodlands in other countries has been recognized and caused political and economic disruptions.

As compared to conditions in central Wisconsin that favor the development of local epidemics, similar conditions in other areas may differ in ways to limit or to slow the spread of oak wilt. Even in Wisconsin, the disease has not invaded northern oak stands and has moved slowly and intermittently among oaks of southern Wisconsin. Various explanations have been proposed.

Stand Composition

First, in many north central and eastern states, discontinuous stands of widely-spaced and uneven-aged oaks exist among mixed hardwoods. Even within the red oak groups, susceptibility of eastern oaks may be less than that of northern pin oak. Thus, single tree infections may fail to spread or only spread slowly, intermittently, or temporarily from tree to tree. Under such conditions, even though climate may permit oak wilt development and vectors may be present, inoculum may be limited. Wounding will vary greatly with the time, place, and amount of human activities.

Stand History

Oak management practices differ in that selective cutting generally has replaced clear cutting. Vegetative regeneration does not create extensive, even-aged, dense oak stands. Regeneration of oaks may require several years. Meanwhile, seedling regeneration will not soon form a dense network of oak roots and root grafts among young widely-spaced oaks will be limited.

Soil Types

No doubt, the physical arrangement and functions of tree root systems vary with soil type, structure, and composition. Root grafting may be limited in many areas (Jones and Phelps 1972). In Wisconsin, limited examinations of root systems of oak growing in lowlands on silt loam soils showed deeper root systems and limited root grafting. Tree-to-tree spread

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was slow between and among trees in oak savannas and oak openings. But such observations are modified by stand composition and other factors already mentioned.

Inoculum

Unless widely scattered, infected oaks give rise to active oak wilt pockets. Inoculum for additional infections will be limited and temporary.

Vectors

Oak wilt vectors are relatively inefficient and ineffective. The nitidulid beetles, for example, need a source of ready, abundant inoculum (sporulating mats) during the periods of high oak susceptibility (at the right time) to be deposited in fresh wounds (at the right place) under favorable conditions of moisture and temperature. Apparently, this critical combination and sequence of events occur infrequently.

Environment

Environmental conditions, both climatic and seasonal, appear to favor disease development if and when infections occur. Of course, periods of high host susceptibility vary and shift with seasonal and periodic conditions.

CONCLUSION

In conclusion, epidemiological evidence enables the formulation of effective control measures which combine both preventive and curative measures.

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Epidemiology of Oak Wilt in Texas

David N. Appel

The epidemic of oak wilt in Texas, caused by *Ceratocystis fagacearum*, has been extensively studied and compared in detail to oak wilt epidemics in the 21 other states where the disease is found. The pathogen itself appears to be no different in Texas. Other components of the epidemic, such as host susceptibility and climate, are unique, resulting in unanticipated difficulties in controlling the disease. The semi-evergreen live oaks in Texas respond to infection by *C. fagacearum* with greater variability than the deciduous red and white oaks, influencing the rate of disease progress in individual trees. Species composition and the limited opportunities for inoculum formation on red oaks, which comprise only a minor component of most oak savannahs in Texas, greatly influence the rate of disease progress in populations of trees. The unique environmental conditions in Texas, including climate and length of growing season, influence inoculum formation, inoculum viability, transmission by insect vectors, and tree susceptibility. The Texas oak wilt epidemic has provided an excellent exercise in comparative epidemiology, but there are still many facets of the disease cycle that are poorly understood. Until a better understanding of the disease is attained, significant progress in improving current disease control technology will be delayed.

INTRODUCTION

A plant disease epidemic occurs when a pathogen spreads to affect large proportions of a host population in a relatively short period (Agrios 1988). The study of conditions that allow an epidemic to occur is called epidemiology. A thorough understanding of those conditions will lead to better disease management and reduced losses of trees. Since epidemics in forest situations are highly complex processes, their analyses can be facilitated by categorizing key components of the process into four groups; 1) host influences, 2) pathogen influences, 3) environmental influences, and 4) temporal influences. These components do not operate independently. Each may alter or modify the effects of another, so that a complex web of interacting components controls disease progress. Occasionally, the components of the web interact to create conditions conducive to losses of enormous numbers of host plants; this is the case for the Texas oak wilt epidemic.

Oak wilt was first discovered in Texas in 1961 (Dooling 1961). Although the disease may have been present in Texas since the early 1930's (Taubenhaus 1934), the extent of the problem and appreciation for the impact have been realized only in the past 15 yr (Appel and Maggio 1984, Lewis 1977). Oak wilt also occurs in 21 other states, where intensive survey, research, and management programs have been carried out since the original description of the pathogen, *Ceratocystis fagacearum* (Bretz) Hunt, from Wisconsin in 1944. The results of these previous efforts are useful for analyzing the comparative epidemiology of oak wilt in Texas. Presumably, such an analysis should clarify how oak wilt control might best be managed. The abundance of literature pertaining to oak wilt epidemiology in

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states other than Texas is summarized in excellent reviews, including those by MacDonald and Hindal (1981) and Gibbs and French (1980); these should be consulted for a more detailed survey of oak wilt research. In the following discussion, conditions known to be conducive for development of oak wilt into epidemic proportions in other states will be compared to those in Texas.

THE INFLUENCE OF HOST COMPOSITION ON OAK WILT DEVELOPMENT

Host Susceptibility

In the initial description of oak wilt, a direct relationship between *Quercus* taxonomy and susceptibility to oak wilt was noted (Henry et al. 1944). Red oaks, in the subgenus Erythrobalanus, are extremely susceptible to colonization by the fungus. Complete wilting and subsequent death of red oaks, e.g. northern red oak (*Q. borealis* Michx.), Shumard oak (*Q. shumardii* Buckl.) and pin oak (*Q. palustris* Muenchh.), occur in a matter of weeks following initial symptom appearance. In contrast, the white oaks (subgenus Leucobalanus) are extremely resistant to colonization by the pathogen. Infected white oaks such as white oak (*Q. alba* L.) and post oak (*Q. stellata* Wangenh.) decline slowly over a period of years, usually recovering with only limited loss of a few branches (Parmeter et al. 1956). These same relative susceptibilities are true of deciduous red and white oaks in Texas. However, semi-evergreen live oaks (*Q. fusiformis* Small and *Q. virginiana* Mill.) are the most seriously affected of the Texas *Quercus* population and they are an exception to the clear association between taxonomic status and susceptibility to the pathogen. Most infected live oaks die within 3 - 6 months following the initial appearance of symptoms, but a significant proportion, averaging 10 - 20%, survive indefinitely in various stages of crown loss (Appel 1986, Appel et al. 1989).

Since they possess characteristics common to both Erythrobalanus and Leucobalanus, the subgeneric classification of live oaks is difficult. Acorn fertilization and maturation in live oaks is similar to white oaks; acorns on white oaks germinate the season they first develop. For this and other reasons, live oaks are usually classified with the white oaks (Muller 1961, Tillson and Muller 1942). Alternatively, the live oaks have thick-walled, rounded summerwood vessels and sparse production of tiny, bubble-like blockages called tyloses in the water-conducting xylem. These are typical red oak characteristics, and lead to confusion in classification (Miller and Lamb 1985, Williams 1939). These common xylem characteristics also may explain the susceptibility of live oaks and red oaks to the oak wilt pathogen. In contrast to the complete susceptibility of red oaks, disease centers in Texas live oak stands invariably have debilitated, surviving trees existing in the older parts of the patch of dead trees. The presence of mechanisms responsible for survival of live oaks in Texas warrants further investigation.

Inoculum Production and Insect Transmission

The importance of *Quercus* taxonomy to the epidemiology of oak wilt goes beyond mortality rates in individual trees. Abundant inoculum production by the pathogen is known to occur only on infected red oaks (Gibbs and French 1981). Spores, consisting of both conidia and ascospores, grow on the surface of mycelial mats that form beneath the bark of infected red oaks. Because of their sweet smell and suitability as a food source, the mats attract sap feeding nitidulid beetles (Coleoptera: Nitidulidae). After feeding and breeding on the mats, contaminated nitidulids emerge and disperse. Spore-bearing nitidulids may transmit the fungus by feeding on wounds on healthy oaks. Presumably, in this manner the fungus is transmitted to live oaks over long distances (Dorsey et al. 1953, Juzwik and French 1983).

There is also evidence for the transmission of *C. fagacearum* by bark beetles (*Pseudopityophthorus* spp.) that do not require fungal mats for inoculum acquisition (Rexrode and Jones 1970). However, the preponderance of evidence implicates nitidulids as the primary vectors throughout much of the range of the disease. In Texas, fungal mat formation is known to occur only on diseased Spanish oaks (*Q. texana Small*) and blackjack oaks (*Q. marilandica*

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Muenchh.); both are typical deciduous red oaks (Appel et al. 1987).

The pattern of fungal mat production in Texas is similar to formation on deciduous oaks throughout the range of the disease (Table 1). Mats do not form on dead trees, nor do they form on trees that are in the early stages of colonization. The environmental conditions required for mat production include cool temperatures and high moisture, so in Texas they cannot be expected to be present from May through October of a typical Texas summer. Table 1 lists rates of mat production on ten diseased Spanish oaks. These trees were selected in September for observation because they exhibited oak wilt symptoms in their crowns but still had green cambial layers, indicating they were not yet dead.

Those trees with 100% symptom development in September were dead and colonized by *Hypoxylon atropunctatum* the following spring. Trees with only limited symptom development resprouted the following spring, eventually dying with no mat formation. Apparently, colonization must be in a proper stage for inoculum production to occur, therefore limiting the numbers of diseased trees that will produce mats and the subsequent development of new disease centers. This weakness in the disease cycle serves to limit the rate of epidemic increase of the disease, and is consistent with observations of mat production on red oaks elsewhere (Curl 1955).

Root Transmission

Fungal mats do not form on infected white oaks. More importantly to the Texas oak wilt epidemic, they also fail to form on infected live oaks. Pathogen spread among live oaks is believed to be limited to transmission between diseased and healthy trees via functional root connections. Root connections may form when the roots of two adjacent trees grow together to produce a union of xylem tissues, and are well documented as a means of transmission for *C. fagacearum* (Skelly and Wood 1974, Yount 1955). The importance of root graft transmission is believed to vary within the range of *C. fagacearum*, but in some locations, this means of transmission is responsible for a majority of the losses. In Minnesota, root graft transmission among deciduous red oaks causes the disease to occur in discrete "centers," or foci, which expand on the perimeters at about 7.5 m/yr (Gibbs and French 1980). Expansion rates in Texas live oak were found to be much greater, at an average of 11 - 16 m/yr with longer maximum distances of 40 m/yr occurring commonly (Appel et al. 1989). Consequently, disease foci in Texas on live oak can involve thousands of trees and reach dozens of hectares.

The rapid rate of spread and large sizes of disease foci in Texas live oak are believed to be a result of grafting combined with an additional type of root connection. Live oaks are unique because they form rhizomes for vegetative propagation through root sprouting (Muller 1951). If the ramets maintain common root connections through maturity, they will provide an additional mechanism for transmission of *C. fagacearum*. As in other states on deciduous trees, the pathogen in Texas is consistently transmitted through adjacent live oaks in a highly predictable manner so that discrete, well-delineated foci are formed (Appel et al. 1989). Satellite centers do not occur in the immediate vicinity of oak wilt foci in homogenous live oak stands because there are no known insect vectors in live oak that will transmit the fungus beyond distances expected of the root connections.

Spatial Dynamics

In other states where oak wilt occurs, a standard convention for expected distances of pathogen transmission was adapted. The distance for root graft transmission was not expected to exceed 17 m annually, and was termed "local" spread (Gibbs and French 1980). Long distance, overland spread of the pathogen by nitidulids was accepted to be any observed occurrence of the pathogen 17 m or more beyond the presumed source of inoculum. These two types of transmission determine the relative risk of infection for trees in the vicinity of oak wilt.

In Texas, live oaks growing immediately adjacent to another diseased live oak are virtually certain to become infected (Appel et al. 1989), while a Spanish oak will not. Spanish oaks are presumably less likely to be grafted to a live oak. Despite their greater susceptibility, they are often, but not always, left

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Table 1. Symptom development and fungal mat production during 1983 on 14 Spanish oaks infected with *Ceratocystis fagacearum* in central Texas.

		Observation/Month[1]								
Tree #	**Symptoms[2]**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	50%	-	-	-	-	-	-	-	-	-[3]
2	70%	-	-	-	-	-	-	-	-	-[3]
3	100%	-	-	-	-	-	-[4]	-[4]	-[4]	-[4]
4	80%	-	-	-	-	-	-	+	+	-
5	90%	-	-	-	-	-	+	+	+	+
6	95%	-	-	-	-	-	-	-	+	-
7	95%	-	-	-	-	-	-	+	+	-
8	5%	-	-	-	-	-	-	-	-	-[3]
9	80%	-	-	-	-	-	-	-	-	-[3]
10	100%	-	-	-	-	-	-[4]	-[4]	-[4]	-[4]
11	30%	-	-	-	-	-	-	-	-	-[3]
12	90%	-	-	-	-	-	-	-	+	-
13	5%	-	-	-	-	-	-	-	-	-[3]
14	50%	-	-	-	-	-	-	-	-	-[3]

[1] - indicates no mats were found on the tree;
+ indicates the presence of fresh, functional mat production.

[2] All trees were chosen on the basis of exhibiting foliar symptoms of oak wilt but retaining green cambial tissues; the figure in this column refers to the proportion of symptomatic foliage to healthy, green foliage.

[3] These trees were resprouting.

[4] These trees were exhibiting *Hypoxylon atropunctatum* stromata on the surfaces of the lower trunk of deciduous red oaks (Appel et al. 1987).

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unaffected following passage of the disease front through a mixed stand. The perimeters of oak wilt foci in live oak stands are easily defined; there is a sharp contrast between the diseased, symptomatic, and asymptomatic trees. Although oak wilt centers are commonly very large, they are often very few in number. This is apparently because long distance transmission into a new area is a relatively rare event when there is a lack of red oaks providing inoculum for long distance spread. Disease patterns of this sort are common in the coastal plain, southeastern portion of the oak wilt range in Texas, as well as in other locations where red oaks are sparse.

Disease foci are less well defined and greater in number when significant populations of Spanish and blackjack oaks comprise a stand. Since both of these red oaks support fungal mat formation in Texas, insect transmission causes randomly dispersed patches to occur within the affected vicinity. In areas where there is a great proportion of diseased red oaks, the foci are more diffuse, less distinct, and there is a greater incidence of individual foci with varying distances among them. The pattern of oak wilt in Kerr, Bandera, and farther north in Lampasas and Burnet counties has these characteristics. The potential for inter-species root grafting between live oaks and red oaks is unknown but is presumed to occur occasionally, allowing for both types of pathogen transmission (Epstein 1978). The composition of the *Quercus* population therefore contributes to the spatial distribution, incidence of foci, and the rate at which tree losses are accrued.

PATHOGEN INFLUENCES ON OAK WILT DEVELOPMENT

Pathogen Variability

The reproductive cycle of the oak wilt pathogen strongly influences the rate of epidemic increase and the capacity for the pathogen population to survive and spread. The fungus *C. fagacearum* is a heterothallic Ascomycete, with two mating types required to produce the fruiting structures that characterize the sexual stage. The sexual cycle of the fungus is completed in nature when perithecia and ascospores form on the fungal mats on diseased red oaks. The mats are fertilized for perithecial formation by insects carrying conidia, the asexual spores, from mats on a tree colonized by one mating type (A or B) to mats of the opposite type forming on another tree. Mating type is easily identified in the laboratory, and is one of the few "markers" currently available to study and observe the behavior of specific *C. fagacearum* isolates in the field. Both mating types are usually found in equal proportions in regions where surveyed (True et al. 1961). However, both mating types are rarely isolated from an individual tree. Each type of spore, i.e. ascospore or conidium, is capable of transmitting the disease, but the relative importance to long distance transmission is unknown (Engelhard 1956).

Although little direct genetic work has been done on *C. fagacearum*, some variability in the pathogen population has been noted. Differences in culture pigmentation in the laboratory have been observed, and some albino mutants identified. Further isolate variation was found in sporulating ability and physiological activity (Barnett 1956). The relatively recent appreciation for the extent and severity of the Texas oak wilt epidemic stimulated speculation that the pathogen had changed in some manner and subsequently initiated the apparently new outbreak in Texas. There was no evidence for increased heat tolerance in the Texas pathogen population when compared to isolates from other parts of the disease range (Lewis 1985c).

In the laboratory, Texas isolates are indistinguishable from isolates originating in other states. The pathogenicity of a West Virginia isolate was compared to the abilities of seven Texas isolates to cause symptom development in inoculated, containerized live oaks (Peters 1985). There was a fair degree of variation in pathogenicity of the isolates, as was expected from the results of previous inoculation experiments (Cobb and Fergus 1964). The West Virginia isolate had a slower rate of disease progress after 60 dys. than all of the Texas isolates except one, from Bell Co. In inoculation trials on field grown red oaks growing in South Carolina, Texas isolates produced a slower rate of disease progress but eventually caused the same degree of symptom development as *C. fagacearum* isolates from South Carolina, West Virginia, and Wisconsin.

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Although no consistent trends in pathogen variation have been established, the results of these studies provide no evidence for any significant difference in the pathogen population currently causing the Texas oak wilt epidemic.

Origin of the Pathogen

The origin of *C. fagacearum* in the United States is unknown. Although first discovered in Wisconsin in 1941, there is evidence that outbreaks of oak wilt occurred as early as the 1880s. Likewise, there is no information on the origin of *C. fagacearum* in Texas, although evidence indicates that oak wilt was present in Austin at least 30 years prior to the first report in 1961 (Taubenhaus 1934). There is insufficient information to determine the extent or rate of increase of oak wilt in Texas during the first 30 years of research on the disease elsewhere. By 1985, both mating types of the pathogen were evenly distributed through most of the known range of the disease (Appel et al. 1985), indicating that the Texas *C. fagacearum* population is not a new introduction. The expansive range further indicates that the pathogen may occur further south in Mexico and Central America, where hundreds of additional live oak species exist. But, the pathogen has yet to be identified in those countries.

ENVIRONMENTAL INFLUENCES ON OAK WILT DEVELOPMENT

Inoculum Formation and Vector Acquisition

As previously stated, fungal mat formation depends on the stage of colonization of the tree and the occurrence of cool, moist conditions. Conditions in Texas appear to be ideal for mat formation beginning in October or November and extending through the end of May. Populations of air-borne nitidulid beetles in Texas are highest during March through May, the latter part of the period of peak mat formation (Appel et al. 1986, 1987). Nitidulid populations then decline during the summer months but may increase slightly in the fall. Beyond this information, specific details of the behavior of nitidulids and the relative contribution they make to a growing epidemic are unavailable. There are at least six nitidulid species that may act as vectors in Texas, with *Cryptarcha concinna* Melsheimer and *Colopterus maculatus* (Erichson) being the most likely vectors. These species were trapped in greatest numbers in Texas and have a history of involvement with oak wilt in other states (Appel et al. 1986). These two species comprised the majority of beetles trapped in Texas and found to be contaminated with *C. fagacearum*. Most of those beetles found carrying the pathogen were trapped in April, although one contaminated beetle was trapped in July (Appel et al. 1990). The contamination of nitidulids in the spring, however, is probably most important because this is also the period of greatest susceptibility of live oaks, as demonstrated with artificial inoculations of field trees (Appel et al. 1986).

Tree Susceptibility and Fungal Colonization

Texas is the southernmost state to report the occurrence of oak wilt. Prior to detecting the extent of the epidemic in Texas, the southern expansion of *C. fagacearum* was considered to be limited by high temperatures (Schmidt 1978). Growth of the pathogen was consistently shown to be inhibited by temperatures above 30°C in the laboratory (Lewis 1985c, Merek and Fergus 1954). The same was true of the ability of the fungus to induce symptom development in growth chamber inoculation trials in young trees (Houston et al. 1965). In spite of this heat sensitivity, the fungus in Texas has been found as far south as Atascosa County south of San Antonio, where temperature extremes often exceed those considered to be limiting to growth of the fungus. The pathogen probably overcomes the influence of high temperatures underground in the root collars and roots of the tree. Lewis (1985c) measured sapwood temperatures in root collars of live oaks in Texas and found them to be favorable for growth of the fungus during the warmest months of the year.

The role of high temperatures may be important in the 10 - 20 % survival rate of live oaks in Texas, although few of the remaining trees survive in good condition. In South Carolina, a normally susceptible red oak population consisting of turkey oaks (*Q.*

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Table 2. Inoculation dates, infection courts, and disease development[1] on live oaks growing near Burnet, TX and inoculated with *Ceratocystis fagacearum*.

Innoculation Date	Infection Court	Percent Disease	Latent Period[2]
May 31	Branch	17	67
	Trunk	100	55
	Root	100	55
September 9	Branch	0	-
	Trunk	17	229
	Root	33	294
January 3	Branch	0	-
	Trunk	17	203
	Root	17	203
March 15	Branch	0	-
	Trunk	83	131
	Root	50	131

[1] Disease development is represented by "Percent Disease" and was estimated by observing the proportion of defoliation in tree crowns. This figure is the average of 10 trees.
[2] The latent period refers to the numbers of dys. between inoculation and initial symptom appearance.

laevis Walt.) was not affected by oak wilt to the extent expected. High temperatures are believed to slow colonization and inhibit symptom development so that normally susceptible trees are able to respond and survive (Tainter 1986). In Texas, this might be applicable to those trees in the early stages of colonization in May and June. Temperatures are sufficiently high to eradicate the fungus from the upper crown in July and August, leaving portions of the tree disease-free. Isolation of the fungus from branch tissues in the summer is extremely difficult (Appel and Maggio 1984). Long-term survival would then depend on internal resistant mechanisms in live oak being able to inhibit further colonization by the fungus. This scenario is one of many that would be useful for investigating the causes of tolerance in live oak to the oak wilt fungus.

Temperatures throughout most of the oak wilt range during winter in Texas are unlikely to be limiting to growth and survival of the fungus. Spread of the pathogen through live oak root systems probably occurs during most months, accounting in part for the rapid rates of spread observed in Texas. Infection by nitidulid beetles, however, is likely to be limited to spring. Disease development on artificially inoculated trees growing on central Texas rangelands was clearly greater during the spring (March and May) than during fall or winter (September or January) (Table 2). Numbers of diseased trees were greater and incubation times shorter during spring.

CONCLUSIONS

Oak wilt is widely recognized in Texas as one of the most serious plant diseases in the state. This unusual recognition is due to the economic value of live oak (Martin et al. 1989) and the impact the disease is having on urban forests in Austin, Waco, Dallas/Ft.

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Worth, and dozens of other cities in central Texas. Oak wilt is not usually considered to be a significant urban tree disease, but in Texas has achieved much notoriety as a limiting factor to the value of live oak in the landscape. Since most live oaks in the urban forests are native trees left after development, the epidemiology of the fungus in urban populations is similar to that observed in forests and rangelands.

In terms of losses, *C. fagacearum* in Texas may be responsible for greater tree mortality than in any other portion of the disease range in the United States. In contrast, oak wilt has not caused the losses of trees originally anticipated in the deciduous forests of the Mid Atlantic, Midwestern, and North Central states. There is no evidence to indicate that any significant changes have occurred in the pathogen population to account for the high losses in Texas. Rather, the influence of stand composition, affected *Quercus* species, and temperate climate are believed to be responsible. The structure of the live oak population in Texas has undergone dramatic changes in the past 150 yr due to fire control, overgrazing, and selective tree management, creating conditions highly conducive to development of an epidemic (Appel and Maggio 1984). Compared to the high incidence of oak wilt in central Texas (see range map on inside back cover), the lack of oak wilt in the diverse east Texas piney woods remains a mystery. There are large components of southern red oak (*Q. falcata* Michx.), water oak (*Q. nigra* L.), and live oak in east Texas, so the pathogen may yet spread to that portion of the state. Likewise, with long-term changes in forest composition in the southwestern and Pacific regions, the Texas oak wilt epidemic may be an indicator of the destruction oak wilt may have on new *Quercus* species growing in forests considered to be safe from the disease.

Oak wilt management programs are traditionally designed to reduce inoculum, prevent infection courts, and inhibit transmission through root connections. With the exception of intravascular injection of trees with fungicides, the Texas approach to oak wilt management is consistent with those methods used for the past 50 yr in other states. However, a great deal of modification of specific control techniques has been necessary to adapt them to the epidemic conditions found in Texas (see Cameron and Billings, these proceedings). For example, breaking root connections in Texas to prevent "local" spread of the pathogen requires placement of the trench at least 100 ft beyond the symptomatic tree on the focus perimeter, rather than shorter distances used in other states. In spite of those adaptations, disease control efforts in Texas are still inadequate. As studies on the epidemiology of oak wilt continue, existing management techniques will undergo further refinement and improvements will be made in our ability to reduce tree losses.

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Insects and the Epidemiology of Oak Wilt

William Merrill
David W. French

The pattern of establishment of new infection centers of oak wilt, caused by *Ceratocystis fagacearum*, indicates an insect vector carried by the wind until it impacts on the edges of stands or openings, on dominant trees protruding above the forest canopy, or onto the windward sides of ridges. Nitidulid beetles (Coleoptera: Nitidulidae) may function as vectors for both short-range and long-range spread, but are not the sole vectors. The oak bark beetles (Coleoptera: Scolytidae) also can function as vectors. Ambrosia beetles (Coleoptera: Scolytidae) may be occasional vectors, especially if the pathogen becomes established in their mycangia. Based on their life cycles, certain buprestids (Coleoptera: Buprestidae) and cerambycids (Coleoptera: Cerambycidae) also may occasionally serve as vectors. Best evidence at present suggests the existence of several relatively inefficient vectors. However, after 40 years of oak wilt research, there still is no convincing proof as to the relative importance of any of these insects in the establishment of new oak wilt infection centers.

INTRODUCTION

In the early years of the chestnut blight epidemic, Metcalf and Collins (1911) proposed that a 10-20 mi-wide zone of chestnuts be eradicated close to the killing front, and that outlying foci then be located and eradicated. The State of Pennsylvania formed and funded the Pennsylvania Chestnut Tree Blight Commission whose responsibilities were to study the blight and to recommend means of controlling it (Pearson et al. 1912). At the 1912 Blight Conference, Dr. F. C. Stewart (*ibid* pp. 40-45) of the New York Agricultural Experiment Station Geneva protested the Metcalf-Collins plan, reviewing what was known about this disease and fungus diseases in general and, because the major means of dissemination still were only speculative, concluding that at that time there was no known way of controlling the disease, finally stating:

> "It is better to attempt nothing than to waste a large amount of public money on a method of control which there is every reason to believe cannot succeed."

W. A. Murrill (*ibid* pp. 194) concurred, but was verbally assaulted and ridiculed (*ibid* pp. 195-201) by one of the Pennsylvania Deputy Commissioners of Forestry (to the repeated applause of the large audience), and Pennsylvania went on to adopt a control program involving the cutting-out of all infection centers in the western portions of the state; this program was followed by similar efforts in Virginia and West Virginia (Sargent 1914). In retrospect, Stewart and Murrill obviously were correct. It was only after the Blight Commission had submitted its final report that Heald and Studhalter (1914, 1915) confirmed wind dissemination of the ascospores and that the pathogen could be vectored by birds, and Studhalter and Ruggles (1915) proved several different types of insects carried the pycnidiospores. Perhaps it was the lingering remembrance of this fiasco that caused many early investigators of oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, to focus on means of spread of the fungus. Yet in spite of this, state-wide oak wilt control programs were initiated with little or no knowledge of how and when the fungus was disseminated!

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INSECTS AND OAK WILT MATS

Many early investigations concentrated on the insects and other organisms that visited oak wilt mats, as these were the most obvious potential sources of inoculum. Such studies implicated a large array of genera and species in several insect orders, including Hymenoptera, Diptera, Coleoptera, and Collembola; some of this is reviewed and summarized in the monograph by True (et al. 1960). Squirrels also were implicated (Himelick and Curl 1955).

The conclusions of nearly all of these studies are extremely questionable. Insects were placed on sporulating cultures of the pathogen for various lengths of time, and then caged over fresh wounds on trees. Is it any surprise that the trees wilted? The same results could have been obtained by rolling small stones or buckshot on the sporulating cultures and then placing the stones or buckshot in the wounds. Virtually none of these studies proved that the insects actually could be or were vectors in nature. The studies on squirrels as vectors are similarly suspect.

To serve as a vector from a sporulating mat, the insect would have to frequent the mats, acquire the fungus, fly to a healthy oak, and then in some manner deposit inoculum into an infection court. Either the insect would have to create the infection court, i.e., make some type of injury through the bark into the xylem, or it would have to find some type of a fresh wound that could serve as an infection court.

The nitidulid or sap-feeding beetles (Coleoptera: Nitidulidae) satisfied all of these requirements, and much attention was focused on them. Also, trees wounded in the spring commonly became infected both in Pennsylvania (Jeffrey 1953) and Wisconsin (McMullen et al. 1954). Morris et al. (1955) released large numbers of nitidulids, tagged with radio isotopes, in the centers of plots surrounded by concentric rings of freshly wounded oaks at various distances from plot center. Relatively small numbers of the beetles were collected on the wounds. Two of these beetles were collected the following day across a valley on another ridge a mile away!

More recent similar studies in Minnesota also have shown that nitidulids are important vectors (Juzwik and French 1983, 1986, Juzwik et al. 1985). These insects are attracted to relatively fresh wounds on virtually any plant species, as well as to such things as food on picnic tables, as one of their common names, "picnic beetles", suggests. In trapping studies in Pennsylvania, overripe bananas and stale beer were the best attractants. When leaving oak wilt mats, nitidulids appear to fly in a random pattern, and even if they are carrying propagules of *C. fagacearum*, they may never come in contact with an oak tree.

Nitidulids cannot wound trees, but require fresh wounds. In Minnesota, oaks are susceptible to inoculation by nitidulids only for a short time in the spring when fresh oak wilt mats are available (Juzwik et al. 1985). Within six dys. after the bark covering oak wilt mats splits open, the potential for insect vectors carrying *C. fagacearum* decreased rapidly (Ruetze and Parameswaran 1984). In Minnesota, these dates vary from year to year and also differ depending upon the location of the site from north to south. In seven locations with 158 oaks, infection occurred only in trees wounded in late May and early June. Of over 5,000 oaks wounded from 1953 to 1983, infection occurred as early as 1 May and as late as 15 June (Juzwik et al. 1985). Of 322 wounded trees upon which the wounds were painted, none became infected. If oaks are unavoidably wounded in May or June, the wounds should be painted with a nontoxic paint. There are no records of wounded trees becoming infected if the wounds were painted.

If nitidulid beetles are carrying spores of *Ceratocystis piceae* (Munch) Bakshias well as spores of *C. fagacearum*, the oak wilt fungus will not cause infection on a wounded healthy oak (Ruetze and Parameswaran 1984). Furthermore, *C. piceae* is better adapted for insect dissemination and thus insects departing wilted trees would be carrying primarily inoculum of *C. piceae* (Ruetze and Parameswaran 1984).

Ceratocystis fagacearum was isolated from the water washings of seven of 1,043 free-flying nitidulids, one collected in April and the others from 18 May to 19 June (Juzwik and French 1986). *Ceratocystis piceae* was similarly recovered from seven nitidulids collected over a three-week period. In another area, *C. fagacearum* was recovered from 80

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and *C. piceae* from 17 of 98 nitidulids. From 30 to 76,000 spores of *C. fagacearum* were estimated to be present on the beetles (Juzwik and French 1983). Thus, by carrying spores of *C. piceae*, nitidulids may play a role in the natural biological control of *C. fagacearum*.

Free-flying nitidulids were rarely found carrying *C. fagacearum* in late summer and autumn in Minnesota. The pathogen was recovered from only two of 1,145 beetles collected between 30 August and 22 October (Juzwik and French 1986). None of 11 oaks wilted when nitidulids from these collections were caged over wounds. However, two of five oaks wilted when wounds were inoculated with water washings from similarly-collected beetles (Juzwik and French 1986). Approximately 3,300 nitidulids marked with fluorescent dyes were released near wounded oaks in September and October. Marks indicating insect visitation were found in wounds on eight trees (Juzwik and French 1986). Although nitidulids might transmit *C. fagacearum* during late summer and autumn, this probably is rare because the insects are seldom attracted to fresh wounds on oaks in the fall months.

Although many forest pathologists held that nitidulid beetles were the major vectors of the fungus, serious nagging questions remained unanswered. Mats rarely formed in Missouri, Arkansas, and Oklahoma, yet oak wilt continued to spread. In Pennsylvania between 1955 to 1968 there were six drought years when mats rarely formed, yet the number of new oak wilt infection centers increased at a constant rate irrespective of the frequency of mat formation. Oaks without discernible fresh wounds also became infected and died. These occurrences raised questions regarding the means of long-distance spread.

In oak wilt jargon, long-range spread meant the establishment of new foci of infection; short-range spread meant increase in size of existing foci. Yet, there was little consistent use of these definitions. The longest confirmed spread by nitidulids in Minnesota was only 119 m (Juzwik et al. 1985). In contrast, the patterns of apparent spread observed in Pennsylvania and West Virginia, i.e. ridgetop to ridgetop spread in many areas, involved distances in kilometers.

AMBROSIA BEETLES

Ambrosia beetles (Coleoptera: Scolytidae) also were suggested as possible vectors. They have been collected up to 3000 ft in the air (Glick 1939). Numerous genera and species inhabit weakened, dying or dead oaks. In Pennsylvania the oak wilt fungus survived in the lower bole and buttress roots of wilted oaks for up to 3 yr after tree death (Skelly 1968). Several ambrosia beetles commonly colonized the lower bole and buttress roots of wilted oaks during that time, and some carried the fungus in or on their bodies when they emerged from these trees (Batra 1963, Skelly 1968). Surface sterilization of such insects suggested to us that the oak wilt fungus was being carried either in the gut or as a contaminant of the mycangia.

Batra (1963) occasionally found the oak wilt fungus in the mycangia of ambrosia beetles. In preliminary studies, four species of ambrosia beetles, extracted or reared from dead trees, placed for eight hr on cultures of the oak wilt fungus and then caged on trees, caused no transmission. Three species, *Xyleborinus saxeseni* (Ratz.), *Xyleborus xylographus* (Say), and *Monarthrum fasciatum* (Say) would not tunnel into healthy trees. *Xyloterinus politus* (Say) tunneled into healthy trees, but caused no transmission.

In a second study with *X. politus*, 949 insects caged on 191 trees resulted in 246 visible entrance holes, with sap leaking from holes in 110 trees. Although the insects initially carried the fungus and tunneled several mm into the xylem, no transmission occurred (Wertz et al. 1971). These ambrosia beetles seldom retained the pathogen more than 24 hr. In subsequent studies we concluded that the fungus was being carried in the gut, and as soon as the insects started tunneling into trees, the pathogen was voided in fecal pellets.

However, certain ambrosia beetles are known to vector other vascular pathogens. *Xyleborus ferrugineous* (Fabr.) is the principal vector of *Ceratocystis fimbriata* in cacao (Saunders 1965) and *Xylosandrus germanus* (Blfd.) can vector *C. ulmi* (Buchanan 1941). Small numbers of both of these

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Table 1. Coleoptera: Buprestidae, Cerambycidae, and Scolytidae collected in window traps above the oak canopy or on the ground in oak wilt affected stands, Bowers Mt. and Research Hill, Blain, Perry Co., Pennsylvania, 1968.

FAMILY Species	Collected Ground	Collected Air	Dates Collected	Oak Hosts[1]
BUPRESTIDAE				
Agrilus acutipennis Man.	+		6:10 - 6:17	+
Agrilus bilineatus (Weber)	+		5:6 - 5:13	+
Buprestis maculipennis Gory	+	+	7:22 - 8:12	-
Buprestis striata (F.)	+		7:8 - 8:12	-
Chrysobothris spp.[2]		+	7:8 - 8:12	?
Chrysobothris femorata (Olivier)	+	+	6:10 - 7:19	+
CERAMBYCIDAE				
Acmaeops directus (Newm.)	+		6:17 - 7:29	?
Anthophylax malachitius LeConte		+	6:17 - 6:24	?
Anoplodera cordifera (Olivier)	+		7:1 - 7:15	?
Anoplodera nitens (Forster)	+		6:17 - 7:8	+
Centrodera picta (Haldeman)		+	6:17 - 7:15	?
Elaphidion mucronatum (Fabr.)		+	7:1 - 7:8	+
Euderces picipes (Fabr.)	+		6:17 - 7:15	+
Leptura lineola (Say)	+		6:10 - 7:29	+/-
Leptura subhamata (Rand.)	+	+	6:17 - 7:15	-
Monochamus scutellatus (Say)	+		5:13 - 6:24	-
Neoclytus mucronatus (Fabr.)	+		7:15 - 7:28	-
Orthosoma brunneum (Forster)	+		7:29 - 8:5	+
Phymatodes testaceus (L.)	+		5:20 - 5:27	+
Phymatodes varius (Fabr.)	+		5:15 - 6:24	+
Prionus laticollis (Drury)	+		7:8 - 8:5	+
Rhagium inquisitor (L.)	+		5:13 - 5:20	-
Saperda lateralis Fabr.	+		6:17 - 8:5	+
Sarotheses fulminans (Fabr.)	+		5:27 - 6:3	+
Strangalia luteicornis (Fabr.)	+		7:1 - 7:29	-
Urographis fasciatus (Horn)	+		6:17 - 8:19	+

Table continues on the next page.

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Table 1. cont'd.

FAMILY Species	Collected Ground	 Air	Dates Collected	Oak Hosts[1]
Xylotrechus colonus (Fabr.)	+		6:3 - 8:19	+
Xylotrechus sagittatus (Germ.)	+	+	7:8 - 8:19	-
SCOLYTIDAE				
Dendroctonus valens (LeConte)	+		6:3 - 7:1	-
Dryocoetes granicollis (LeConte)	+	+	6:17 - 7:1	+/-
Gnathotrichus materiarius (Fitch)	+		4:29 - 5:6	-
Hylastes porculus (Erichson)	+		5:13 - 5:20	-
Hylastes tenuis (Eichh.)	+	+	5:13 - 8:19	-
Hylastinus obscurus (Marsh.)		+	4:22 - 4:29	-
Hylocuris radis (LeConte)	+		6:24 - 7:1	+
Ips pini (Say)	+	+	6:17 - 7:1	-
Monarthrum fasciatum (Say)	+		6:24 - 8:12	+
Pityophthorus pulicarius (Zimm.)		+	6:17 - 6:24	-
Pseudopityophthorus minutissimus (Zimm.)	+		7:15 - 7:22	+[3]
Pseudopityophthorus pruinosus (Eichh.)		+	6:17 - 7:1	+[3]
Xyleborinus saxeseni (Ratz.)	+		6:24 - 8:12	+
Xyleborus ferrugineous (Fabr.)	+		7:1 - 7:8	+[4]
Xyleborus intrusus Blandford	+		7:22 - 7:29	-
Xylosandrus germanus (Bldf.)	+		5:13 - 5:20	+[5]

[1] + = Known to attack oaks;
+/- = Attacks hardwoods generally;
- = Not known to attack oaks;
? = Hosts unknown

[2] Species previously unrecorded in Pennsylvania

[3] Known vector of *Ceratocystis fagacearum*

[4] Principal vector of *Ceratocystis fimbriata* in cacao

[5] Known vector of *Ceratocystis ulmi*

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species were collected in oak wilt affected stands in Pennsylvania (Table 1). *Xylosandrus germanus* has been collected from Connecticut to Illinois, south to Kentucky; it also occurs in Japan, Korea, China, Taiwan, and Viet Nam (Wood 1982), and thus may be more widely distributed in the United States. *Xyleborus ferrugineous* is one of the most widely distributed, common, and destructive ambrosia beetles in the world, occurring world wide in the tropics and in the Western Hemisphere from Argentina to Massachusetts and Michigan (Wood 1982). If these species can vector other species of *Ceratocystis*, they should be able to vector *C. fagacearum*. Thus, there is reason to believe that certain ambrosia beetles may vector the oak wilt fungus, especially if the pathogen becomes established in their mycangia.

OAK BARK BEETLES

Also to answer the questions posed above, some investigators turned to bark- and wood-boring beetles (Coleoptera: Scolytidae, Buprestidae, Cerambycidae). The oak bark beetles, *Pseudopityophthorus minutissimus* (Zimmermann) and *P. pruinosus* (Eichh.) (Coleoptera: Scolytidae), have habits somewhat similar to those of the elm bark beetle, *Scolytus multistriatus* (Marsham), which make them potential vectors. Buchanan (1958) was the first to demonstrate convincingly that *P. minutissimus* could vector the oak wilt fungus from bolts from infected trees to healthy caged seedlings. Berry and Bretz (1966) later reported that high percentages of the insects emerging from infected wood carried the fungus but postulated that perhaps only small percentages could transmit it.

Rexrode and Jones (1971) then found that both *Pp. minutissimus* in Missouri and *P. pruinosus* in West Virginia carried the fungus when emerging from wood of infected trees in the spring; beetles from 47% of all trees carried the fungus, with from 0 to 33% of the beetles from individual trees carrying the fungus. During two summers of field studies, we were unable to find feeding damage of these insects in Pennsylvania, although we had trapped both species in oak wilt stands. Rexrode and Jones visited us; we felled three healthy red oaks at random and found *P. pruinosus* feeding sites on every branch that we examined. Feeding damage was common and extensive throughout south-central Pennsylvania once we learned to identify it.

Numerous studies indicate that these species are likely vectors, although they are thought to be unimportant in the Lake States. They occur throughout the known distribution of oak wilt. However, small numbers appear to acquire the pathogen from tree tissues, perhaps in part because they tend to breed primarily in the branches where in most years longevity of the pathogen is relatively short. This limited potential vector pool is further reduced because the insects feed on a variety of hardwood species. Thus, they are relatively inefficient vectors, perhaps in part explaining the low r value of oak wilt in comparison to Dutch elm disease (Manion 1991).

Other potential vectors occur in the Buprestidae and Cerambycidae. *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae), the two-lined chestnut borer, has been proven to carry the fungus when it emerges from dead trees (Stambaugh et al. 1955); because adults feed primarily on foliage it may be an occasional vector, but transmission attempts thus far have been negative. Several cerambycids breeding in the branches or buttress roots, such as *Goes* spp. and *Prionus* spp., are potential vectors. They have a two to five-yr life cycle that coincides with the occurrence of "break-over" infections in Pennsylvania i.e., the reoccurrence of wilting trees around the margins of previously-controlled infection centers. Because of their low incidence and long life cycles, they would be extremely difficult organisms with which to do vector studies. However, buprestids and cerambycids have been implicated as vectors of the pathogens associated with vascular mycosis of oak in eastern Europe (Minkevich 1967).

PATTERN OF LONG-RANGE OAK WILT SPREAD

For new infection foci to arise as a result of long-range spread, an insect vector would have to acquire the fungus from a previously-infected tree, fly some distance to a healthy tree, and in some manner introduce the inoculum. To do this, the insect would have to get above the forest canopy. From there, if

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wind speed exceeded the flight speed of the insect, the insect would be blown downwind until it impacted (Kennedy 1975). When wind speed exceeds flight speed, the movement of even the strongest insect flyers is predominantly downwind (Kennedy 1975). This flight would be within a few degrees, perhaps 5-10°, of the mean wind direction during the time of flight. Thus, most long-range dissemination would be more or less passive; even though the insect might be beating its wings, its flight would be controlled primarily by wind speed and direction. This factor has been ignored in most oak wilt vector studies.

In several years' observations in Minnesota north of the Twin Cities, including three yr where we did a 100% ground survey of several square miles of the North Oaks residential development, single tree infections usually occurred at the edges of stands or openings or, if they occurred within the stand, then the initially infected tree usually was a dominant tree. Dominant trees often were the first trees infected in Wisconsin (Riker 1951), Iowa (Brinkman 1952), and Pennsylvania (Craighead and Morris 1952). Furthermore, recovery of both A and B strains of the pathogen from branches in the crown, but not in other parts of tree, indicated inoculation by branch-feeding insects (Boyce and Garren 1953).

In Pennsylvania the "oak wilt hot spot" was the southwestern section of the Ridge and Valley Province. This area consists of a series of more or less parallel, but sometimes deeply folded, shale and sandstone mountain ridges with intervening limestone valleys. In south-central Pennsylvania at the Maryland border these ridges bear about 20°, then gradually turn eastward so that about 55-60 mi north of the Maryland border they bear 45°. Prevailing winds are from the west-southwest. Early observations suggested that most oak wilt infection centers were on the west sides or tops of ridges, i.e. on the sides facing the prevailing wind. However, Wilhour (1968) found no relationship between aspect and incidence of infection in Pennsylvania and Cones (1968) found no relationship in West Virginia. Yet 20 years of oak wilt records suggested that such a relation did exist in Bedford, Fulton, and southern Huntingdon counties. Bowen and Merrill (1971) plotted all oak wilt sites in three sections of those counties onto topographic maps and analyzed their distribution. When ridge bearing was approximately 20°, i.e. somewhat broadside to the prevailing southwest winds, all infection centers were on the west sides or tops of the ridges except in the vicinity of water gaps or wind gaps. In these gaps it appeared that eddy currents swirled the insects around through the gaps to impact on the east sides of the gaps.

The area in which Cones (1968) worked in West Virginia consisted of many small hills, probably with many swirling eddy currents of wind, thus resulting in no relationship of oak wilt incidence to aspect. In Pennsylvania, as ridge bearing gradually turned to about 45°, i.e., more or less parallel to prevailing winds, oak wilt infection centers were equally distributed on both sides of the ridges (Bowen and Merrill 1982). Wilhour (1968) did his research in such an area, thus finding no relationship of disease incidence to aspect. The pattern found by Bowen fits a theoretical model developed to predict gypsy moth (*Lymantria dispar* L.) dispersal in a ridge and valley system (Mason and McManus 1982).

All evidence indicates that new oak wilt infection centers are initiated by insect vectors carried downwind until they impact on the edges of stands or openings, on dominant trees protruding above the forest canopy, or on the windward sides of ridges.

OCCURRENCE OF POTENTIAL VECTORS IN AND ABOVE OAK WILT STANDS

To be involved in long-range spread, the vector must get above the forest canopy. Little is known about the insect fauna above the forest canopy. In a five yr period in Louisiana, Coleoptera formed the second largest group of insects (after Diptera) caught on screens by aircraft (Glick 1939). Several species of nitidulids were collected up to 3000 ft in the air both day and night. Buprestids, cerambycids, and scolytids also were collected, some up to 5000 ft.

To investigate the insect fauna above the forest canopy, we (Merrill et al. unpublished) developed a modified window trap suspended from a boom attached to the top of a dominant tree (most often a Table Mountain pine, *Pinus pungens* Lamb.) so that the bottom of the trap was approximately level with

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the top of the codominant canopy, with the trap guyed to face into the prevailing wind (Merrill and Skelly 1968). We used similar traps on the ground. Such traps are not quantitative; their trapping efficiency depends upon wind speed and insect mass. Insects may be present but not caught because of the low and continuously varying trapping efficiency. Therefore, no conclusions may be drawn about the insect collections except that the insects were present in the locations on the dates that they were collected.

In 1968 we made 12-hr collections, day and night, for three dys. weekly from early May until late August. Thousands of insects were collected; nearly all except buprestids, cerambycids, and scolytids were discarded. We identified the cerambycids; buprestids were identified by Mr. Findley Negley, entomologist with the Pennsylvania Bureau of Plant Industry; scolytids were identified by Dr. S. L. Wood, Brigham Young University (Table 1). In 1970 we (Heuther and Merrill, unpublished) made 12-hr collections in window traps on the ground, day and night, one day weekly studying only cerambycids. We also used white light and blue light traps, as well as fresh-cut "bait" logs to attract insects. We also placed bolts of oaks that had wilted the previous year into large out-of-doors screened insectaries to rear out insects that had colonized the dead or dying trees (Table 2).

Although numerous cerambycids and several buprestids breed in dead oaks, in most instances the adults feed on foliage or flowers, or on trees already declining due to other agencies, and thus are unlikely to transmit the pathogen to healthy trees even if they acquire it. Some species have yet to be studied.

SIGNIFICANCE

A voluminous literature exists regarding insects associated with the oak wilt fungus. There is little doubt that several different species of insects can vector the pathogen, but to date there have been no studies which document the relative importance of any of these potential vectors, in any given area of the country, in the establishment of new foci of infection. The general impression is that all of the vectors are relatively inefficient. It seems likely that in most instances low percentages of the insects acquire the fungus, and this potential vector pool is further diluted by the fact that most species have very broad host ranges. This inefficient vector-pathogen relationship is why oak wilt was not, and is not, another "Dutch elm disease."

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Table 2. Species of Coleoptera: Cerambycidae collected by various means in an oak wilt affected stand, Research Hill, Blain, Perry County, Pennsylvnia, 1970.

Species	Method of Collection [1]	Months of Collection [2]					Oak Hosts [3]
Acmaeops directus (NBwm.)	traps, light		J	J			?
Aegoschema modesta Gyllenhal	light		J	J			+/-
Alosterna spp.	traps	M	J				?
Amniscus maculus (Say)	blue light			J			+
Amniscus sexguttatus (Say)	light		J	J			-
Anoplodera biformis (Newm.)	traps			J			-
Anoplodera canadensis (Olivier)	traps			J			-
Anoplodera cordifera (Olivier)	traps		J	J			-
Anoplodera nitens (Forster)	traps		J	J			+
Anoplodera proxima (Say)	traps		J	J			-
Anoplodera pubera (Say)	traps			J			-
Anoplodera rubrica (Say)	traps		J	J			+/-
Anthophlax attenuatus (Haldeman)	flying		J				?
Anthophlax malachiticus LeConte	traps	M					?
Asemum striatum (L.)	light	M	J	J			-
Astyleiopus variegatus (Haldeman)	light		J				-
Bellamira scalaris (Say)	traps		J				-
Callimoxys spp.	traps, oak trees	M	J	J			?
Centrodera picta (Haldeman)	traps	M	J				?
Centrodera sublineata LeConte	traps	M					?
Clytus spp.	traps	M	J	J			+
Ecyrus dasycerus (Say)	light		J	J			+
Euderces picipes (Fabr.)	flowers	M	J	J			+
Eupogonius spp.	light		J	J			?
Gaurotes cyanipennis (Say)	traps, oak logs		J				+
Graphisurus fasciatus (DeGeer)	traps, light, oak logs	M	J	J	A	S	+
Graphisurus spp.	light				A		?
Hetoemis cinerea (Olivier)	light		J	J			-

Table continues on the next page.

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Table 2 cont'd.

Species	Method of Collection [1]	Months of Collection [2]						Oak Hosts [3]
Leptostylus spp.	reared from oak logs					A		+
Leptura lineola (Say)	traps, light			J				+/-
Leptura subhamata (Rand.)	traps			J	J			-
Lepturges confluens (Haldeman)	light, blue light			J				-
Megacyllene robiniae (Forster)	traps					A	S	-
Microgoes oculatus LeConte	traps				J			?
Molorchus bimaculata Say	flowers	A	M	J	J			-
Monochamus carolinensis Olivier	blue light			J				-
Monochamus scutellatus (Say)	traps		M	J				-
Neacanthocinus spp.	light				J			?
Neoclytus acuminatus (Fabr.)	traps		M	J	J	A		+
Oberea tripunctata (Swederus)	flying			J				-
Obrium rufulum Gahan	light			J	J			-
Orthosoma brunneum (Forster)	traps				J	A		+
Phymatodes aereus (Newm.)	oak log	A	M	J				+
Prionus laticollis (Drury)	traps				J	A		+/-
Psapharochrus quadrigibus	locust logs			J				-
Psyrassa unicolor (Rand.)	light			J	J	A		+
Purpuricenus humeralis (Fbr.)	blue light, oak logs			J	J	A		+
Ragium inquisitor (L.)	pine logs			J				-
Saperda spp.	light				J			?
Saperda lateralis Fabr.	traps, light			J	J			-
Sarosesthes fulminans (Fabr.)	traps, oak logs		M	J	J	A		+
Strangalia bicolor (Swederus)	traps			J	J			+
Strangalia famelica Newman	flowers				J			+
Strangalia luteicornis (Fabr.)	traps			J	J			-
Tetraopes tetrophthalmus (Forster)	flying				J			-

Table continues on the next page.

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Table 2. cont'd.

Species	Method of Collection [1]	Months of Collection [2]	Oak Hosts [3]
Urgleptes querci (Fitch)	light	J	?
Urgleptes signatus (LeConte)	light	J	-
Xylotrechus colonus (Fabr.)	traps, oak logs	M J J A	+

[1] Traps = collected in three modified window traps placed in a semi-circle; light = collected at white light; blue light = collcted at blue light.
Flying = collected in insect nets while flying in the stand; oak logs = collected crawling on freshly-felled oak logs; flowers = adults collected on various species of flowers in the stand.

[2] Months (April to September) in which species was collected; months in which the insect was most commonly collected are underlined.

[3] + = larvae known to attack living or dead oaks; - = larvae not known to attack oaks; +/- = larvae known to attack various hardwoods, but not specifically oak; ? = larval host unknown.

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Influence of Environment on Survival of the Oak Wilt Fungus

Robert Lewis, Jr.

The oak wilt fungus (*Ceratocystis fagacearum*) is sensitive to high temperatures, but is not limited to the cooler climatic zones in the United States. Recent documentation of epiphytotic levels of oak wilt in Texas suggests that the fungus is capable of existing almost anywhere in the United States where susceptible oaks grow. Texas isolates of the fungus appear to be as sensitive to high temperatures as northern isolates, but oak wilt develops in Texas during the hottest periods of summer. Living sapwood is much cooler than ambient air temperatures in the summertime and supports extensive growth of the fungus. Isolation of the oak wilt fungus is most successful when trees exhibit initial wilt symptoms and least successful when trees exhibit dieback. Lower bole and root tissues are cooler and more likely to harbor the oak wilt fungus than branch and twig samples in the summer. Also, the microclimate is more favorable for oak wilt in healthy tissues than in diseased tissues.

INTRODUCTION

The oak wilt fungus (*Ceratocystis fagacearum* (Bretz) Hunt) has been studied by scientists for about 50 yr. As with other economically and ecologically important plant pathogens, the oak wilt fungus has generated much attention in the United States and abroad. One area of research has dealt with growth and survival of the fungus under different environmental conditions. This area of research has been of particular interest to oak log traders and practicing foresters because wood harboring the fungus may pose a threat to susceptible oak trees in areas not yet exposed to oak wilt. Urban foresters and homeowners also have an interest in this line of research because highly valued urban and community trees may be placed at risk if the oak wilt fungus is introduced into an unaffected area. Knowledge about the effects of the environment on the fungus are useful to researchers and arborists as they develop new oak wilt treatment and prevention programs.

A good understanding of how the environment affects survival, growth, and pathological effects of the oak wilt fungus is vital to successful prevention and treatment strategies. The same knowledge of environmental effects can help scientists predict how the intensity and geographical distribution of oak wilt may change under different climatic conditions, especially global warming.

This report reviews the historical account of oak wilt in Texas, where the climate was once believed to be too hot for *C. fagacearum* survival. It also explores the effects of high temperature and host disease status on survival, growth, and isolation of the oak wilt fungus. Special attention is given to how the fungus is able to survive during the hottest period of summer.

HISTORICAL PERSPECTIVE OF OAK WILT AND CLIMATE

No one knows where or how the oak wilt fungus originated. Dying oaks were reported in Madison,

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Wisconsin, by Wardner in 1881. In 1927, Tiemann described disease symptoms in oak trees that would later be attributed to oak wilt. Henry made the first major breakthrough in oak wilt research when he identified the causal agent and confirmed its existence in Wisconsin, Minnesota, Iowa, and Illinois, (Henry 1944, Henry and Moses 1943). Following Henry's discovery, oak wilt research programs were initiated in much of the northern half of eastern United States. Oak wilt research programs were especially active in the central hardwoods region, northeastern areas with significant oak stands, and in West Virginia. Some of the early research demonstrated that *C. fagacearum* is sensitive to high temperatures and that there is a link between climate, fungal growth, and disease development (Bretz and Morison 1953, Englerth et al. 1956, Merek and Fergus 1954). Early research also showed that survival of *C. fagacearum* in wood is adversely affected by high temperatures and low moisture content (Englerth et al. 1956, Partridge 1961, Spilker and Young 1955).

Survey data, coupled with research results on fungal sensitivity to high temperatures, helped shape conventional thinking on where the oak wilt fungus might or might not be able to survive. Summertime temperatures in much of the South were thought to be high enough to exclude oak wilt (Rexrode and Lincoln 1965, True et al. 1960, Van Arsdel et al. 1975). Oak wilt surveys appeared to support this belief because little or no southward movement of the disease was detected during the 1960s and 1970s (Peacher et al. 1975, Rexrode and Lincoln 1965).

Prior to the discovery of oak wilt in northerly states, a major disease of live oaks was advancing through central Texas. The disease was first observed near Austin, Texas, in 1934, where about 200 live oak trees (*Quercus fusiformis* Small and *Q. virginiana* Mill.) were affected (Taubenhaus 1934). There was a clear pattern of disease expansion to nearby healthy trees (Taubenhaus 1935a), but the infectious agent was not identified. Dunlap and Harrison became interested in the same disease and studied it throughout the 1940s. By 1949, they had detected the disease in 16 central Texas counties, but were unable to identify a primary cause (Dunlap and Harrison 1949). Their detailed description of symptoms was useful to other researchers who would study the disease later. Halliwell looked for the primary cause of this disease in the 1960s. By 1964, the disease had been seen in about 60 Texas counties. A *Cephalosporium* spp. was identified as the cause of a slow decline of live oak trees (Halliwell 1964, 1966). In addition to seeing what appeared to be a slow decline of live oaks, Halliwell reported trees dying rather quickly, as had been reported by Taubenhaus in 1935 and Dunlap and Harrison in 1949, but did not attribute the mortality to *Cephalosporium*. The oak wilt fungus was not linked to the live oak disease in the 1960s.

In 1975, *Cephalosporium diospyri* (Crandall) was reported as the primary cause of the live oak disease in Texas. The oak wilt fungus was ruled out as a possible cause, and it was suggested that the first and only report of oak wilt in Texas (Dooling 1961) had either been an error or the high temperatures of Texas had eradicated the fungus from the state (Van Arsdel et al. 1975). Just two yr later, the oak wilt fungus was isolated, identified, and linked to the historical live oak disease for the first time (Lewis 1977). The oak wilt fungus caused rapid mortality in some live oak trees and a relatively slow progression of the disease in others (Lewis and Oliveria 1979).

The magnitude of the problem was elaborated by Appel and others (Appel and Maggio 1984, Appel et al. 1987, 1990). Appel and his colleagues have confirmed oak wilt infections in about 45 Texas counties and some of the individual oak wilt sites cover several acres. Based on the current amount of oak wilt in Texas and its widespread distribution, one can conclude that the oak wilt fungus has been at work in Texas for many years. Even though we may never know for sure whether the disease reported by Taubenhaus and Dunlap and Harrison was oak wilt, the symptoms they described and pattern of disease spread are similar to what has been reported for oak wilt in more recent years (Appel 1986, Lewis et al. 1983). I believe the live oak disease reported by Taubenhaus in 1934 was oak wilt, caused by *C. fagacearum*.

Based on the epiphytotic level of oak wilt in Texas and distribution of the disease in counties with very hot summertime temperatures, southern climatic zones are not a barrier to the oak wilt fungus. The

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fungus can exist and cause serious damage in spite of high summertime temperatures.

SENSITIVITY TO HIGH TEMPERATURES

Do natural temperatures ever get too high for the oak wilt fungus to survive? Oak wilt in Texas covers a broad geographic range. The summertime temperatures in some of those areas are among the highest in the natural range of susceptible oak trees of North America. One might expect Texas isolates of the oak wilt fungus to be less sensitive to high temperatures than northerly isolates.

Texas isolates of the oak wilt fungus vary in their tolerance to high temperatures. Some isolates survived up to four hr at 42°C and three hr at 45°C but others did not (Lewis 1985). Survival was very low at 45° C. Exposure to 45° C for four hr was lethal to every isolate tested. Northern isolates of the oak wilt fungus did not survive when exposed to 40° C for 24 hr (Fergus 1954). Both Texas and northerly isolates of the oak wilt fungus are killed by abnormally high temperatures.

Growth of the oak wilt fungus is favored by relatively mild temperatures. Even the Texas isolates grow almost three times faster at 26°C than at 30°C and most isolates do not grow at all at 32° C (Lewis 1985). South Carolina isolates of the fungus also failed to grow at 32° C. Cultures grown for seven dy at 24° C and exposed to 32° C showed an abrupt disruption of growth (Tainter 1986). Whether the isolate is from Texas or a northern state, the optimum temperature range for growth of the oak wilt fungus appears to be in the 24-26° C range and growth usually stop at 30-32° C (True et al. 1960).

Isolates of the oak wilt fungus from different geographical regions are inactivated by high temperatures, but the fungus can survive for a period at relatively high temperatures. It seems highly unlikely that temperatures within the natural range of *Quercus* spp. will exceed the lethal level for *C. fagacearum*. For example, will temperatures of 40-45° C be sustained for two to three hr anywhere in the range of oak trees? If so, many of the trees would die from the heat alone.

OTHER ENVIRONMENTAL AND BIOLOGICAL FACTORS

In addition to temperature, moisture content of oak trees and logs affects survival of the oak wilt fungus. Low moisture content lessens the survival rate (Englerth et al. 1956, Partridge 1961, Spilker and Young 1955). However, the fungus can survive in air dried lumber for up to 20 wk (Tainter et al. 1984).

When the oak wilt fungus colonizes a healthy tree, biological and physical changes occur in host tissues. Oak wilt infections disrupt water movement in sapwood which leads to desiccation of leaf and branch tissues. Trees weakened by wilt are susceptible to accelerated secondary infections by other microorganisms. Secondary microorganisms may affect prolonged survival of the oak wilt fungus in sapwood.

Wilting trees are quickly colonized by secondary pathogens and saprophytes. Their growth might be at the expense of the oak wilt fungus. A number of microorganisms that are antagonistic to the oak wilt fungus have been identified in wilting trees (Gibbs 1980, Shigo 1958, Tainter and Gubler 1973, True et al. 1960). For example, in Arkansas, *Hypoxylon* spp. colonized oak wilt trees and depleted carbohydrate reserves, thereby starving the oak wilt fungus (Tainter and Gubler 1973). The same phenomenon was not detected in wilting Texas live oaks (Tainter and Lewis 1982). The physiological condition of wilting trees favors microorganisms that compete with the oak wilt fungus. This process might help reduce the amount of inoculum for possible transmission of *C. fagacearum* to other trees.

Insect vectors expose the oak wilt fungus to an adverse environment. The adverse environment may include other microorganisms, dry air, and hot ambient air temperatures. Nitidulid beetles have been associated with transmission of the oak wilt fungus (Jewell 1956, True et al. 1960). Since transmission by nitidulids is passive, viability of the fungus on the surface of beetles is an important concern, especially where ambient air temperature exceeds the upper limit for *C. fagacearum* growth. In Texas, nitidulids harbored viable inoculum of the oak wilt fungus from March to July (Appel et al. 1990). The highest incidence of the oak wilt fungus on beetles was in

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April, when the temperature was mild and fungal mats abundant. Seasonal variation in temperature probably plays a major role in transmission of the oak wilt fungus by insect vectors.

LIVING TREES PROVIDE A PROTECTIVE ENVIRONMENT FOR THE OAK WILT FUNGUS

In addition to getting its nutrients from oak sapwood, the oak wilt fungus also receives shelter from the harsh external environment. Sapwood and roots of living oak trees create and maintain a favorable environment for growth of the oak wilt fungus. The roots of living trees absorb cool ground water which is translocated through xylem and finally expelled through leaves by transpiration. The constant movement of water through vessels acts as a cooling system for sapwood. Therefore, the internal tree temperatures should be much cooler than ambient air temperatures, especially in the summertime. In addition to the cooling effects of water movement through sapwood, tree bark provides a layer of insulation which gives additional stability to internal tree temperatures.

Oak wilt infections disrupt water movement in trees and the first symptoms are wilting leaves and defoliation (True et al. 1960). Wilted and defoliated crowns do not transpire, therefore water movement in infected and symptomatic trees is less than it is in healthy trees. Less water movement should result in higher interior tree temperatures during hot periods of summer. Sapwood temperatures of diseased live oaks in Kerrville, Texas, were up to 5° C higher at the 1.4-m height of the tree trunk than they were in healthy live oaks in July (Lewis 1985c).

At the root collar level of healthy live oaks, sapwood temperatures remain stable and within the optimum range for growth of the oak wilt fungus even when air temperatures are high. In Kerrville, Texas, the average root collar temperatures ranged from 24.4 to 24.7° C when ambient air temperatures ranged from 25.8 to 36.1° C in July (Lewis 1985c). Interior tree temperatures 1.4-m up the bole of healthy live oaks ranged from 26.1 to 27.7° C in the same period of time. A temperature gradient was evident in both diseased and healthy trees. At some level between the root collar and apex of relatively healthy trees, xylem temperatures are within the optimum range for growth of the oak wilt fungus. At other levels, the temperature is either above or below the optimum range. Some tissues, (i.e., small apical branches and twigs exposed to the sun) may be too warm for fungal growth during summer.

Knowing where the oak wilt fungus resides within trees at various stages of wilt development and under different climatic conditions would be useful when developing a fungicide treatment to target specific sites of infection in individual trees. Taking tissue samples from the likely sites of infection would also improve the accuracy of oak wilt isolations.

ISOLATION RESULTS INFLUENCED BY TIME OF YEAR AND TREE DISEASE STATUS

I would like to conclude this paper by reporting original data on isolation of the oak wilt fungus in Texas during different seasons of the year and from trees with different stages of wilt and dieback development.

Trees with initial oak wilt symptoms, advanced dieback symptoms, and healthy trees within 25 m of oak wilt infected trees were sampled from 1977 through 1985. Tree branch samples were taken from trees in and around 42 discrete oak wilt infection centers in central Texas. Debarked wood samples were submerged in 1.05% sodium hypochlorite for 30-90 sec. and placed on commercial grade potato dextrose agar (PDA) in petri dishes. About 20 wood samples were used from each tree.

Isolation of the oak wilt fungus from branch samples taken from trees with different symptoms are summarized in Table 1. The oak wilt fungus was isolated from 80% of the trees with initial wilt symptoms, but only 14% of the trees with advanced symptoms. In this case, advanced symptoms mean crown dieback. Live oak trees developed extensive crown dieback when they were not completely killed by oak wilt within three months after the initial symptoms. Most of the trees falling in this category developed thin crowns and extensive dieback, but

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Table 1. Isolation of the oak wilt fungus from wilting, dieback, and symptomless oak trees within or adjacent to oak wilt infection centers in central Texas (1977 - 1984, previously unpublished data).

Tree Status	**No. of Trees in Sample**	**% with *C. fagacearum***
Initial wilt	26	80
Dieback phase	7	14
Within 25 m of wilting trees (symptomless)	26	16

Table 2. Isolation of the oak wilt fungus from oak trees with initial wilt and dieback symptoms in confirmed oak wilt infection centers during three periods of the year in central Texas (1977-1985, previously unpublished data).

Season of Year	**No. of Trees in Sample**	**% with *C. fagacearum***
April - June	233	54
July - August	96	26
September - November	195	51

were still living after five years. These symptoms resembled live oak decline as described by Halliwell in 1964, but were actually initiated by the oak wilt fungus. Attempts to isolate the oak wilt fungus from these long-term survivors were unsuccessful.

Over 15% of the symptomless live oaks within 25 m of a wilting tree were infected with the oak wilt fungus (Table 1). These trees later developed wilt symptoms, sometimes in the middle of summer when ambient air temperatures exceeded 37 C°.

Isolation of the oak wilt fungus from branch samples of trees with either initial or advanced wilt symptoms during different seasons of the year is summarized in Table 2. The oak wilt fungus was isolated twice as often during spring and autumn than in summer. The highest rate of successful isolations was in June and the lowest rate in August. According to National Weather Service records for Kerrville, Texas, July and August are the hottest months of the year. However, substantial isolation of the oak wilt fungus was accomplished in spite of the hot weather.

Finally, 36 live oaks with extensive dieback were sampled about one year after initial wilt symptoms to compare recovery of the oak wilt fungus from root and branch samples during summertime. Samples were taken in July and August. The oak wilt fungus was isolated from roots of 36% of the trees and branches of only 6% of the same trees.

Isolation results probably give a number of false negatives because only a small fraction of a tree is taken as a sample for laboratory analysis. The site of infection can be missed, especially if only branch samples are taken. Root and lower bole samples would probably give more accurate results in the summer when trying to confirm oak wilt infections by isolating the fungus.

CONCLUSION

The oak wilt fungus can survive and cause significant disease in some of the hottest parts of the United States. Even though the fungus is sensitive to high temperatures, climate is probably not a major barrier

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to oak wilt distribution. Healthy live oak trees create and maintain an ideal microclimate for growth and pathological effects of the oak wilt fungus during the hottest months of the year. The weak link in the oak wilt disease cycle is probably transmission of the fungus rather than sensitivity to high temperatures.

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Host X Parasite Interactions

F. H. Tainter

Infection of red oaks (*Quercus*; subgenus Erythrobalanus) by *Ceratocystis fagacearum* initiates a sequence of predictable events which usually leads to rapid death of the host. Little is known of the physiological interactions which occur between host and pathogen as colonization proceeds. Host reactions include tylosis and gummosis. After the tree has wilted, mycelial mats and pressure pads may form, thus allowing access to spores by potential vectors. Infection of white oaks (*Quercus*; subgenus Leucobalanus) is associated with all of the above features but in varying degrees of moderation and these species may survive infection. Wilted and recently dead oaks are attractive to a variety of deteriorating organisms.

INTRODUCTION

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is a dramatic disease and makes a lasting impression on anyone who has worked with it. There are few pathogens that kill their tree hosts as quickly as *C. fagacearum*. The process of disease progression within some oak species is fairly well understood histologically, but little is known of the physiologic events which unfold after infection.

INFECTION COURT

Infection may occur through root grafts or wounds. This review will concentrate on the colonization/host response following wound acquisition. Wounds are extremely important for overland spread of *C. fagacearum* as the passive vectors (primarily sap feeding beetles of the family Nitidulidae) feed on sap flowing from such wounds, and contaminated sap is pulled into vessel elements, thus assuring infection. Wounds made during the period of springwood formation are especially important (Jeffrey 1953). The closer the proximity of injured springwood vessels to the wound surface the greater the likelihood of infection. Wounding prior to or after springwood formation generally fails to be associated with infection. In red oaks wounds are susceptible for as long as eight dys., while in bur oak (*Quercus macrocarpa* Michx.) wounds are susceptible for 18-24 hr, depending on the weather (Ohman et al. 1959). Artificial wound inoculations of black oak (*Q. velutina* Lam.) have produced successful infections in roots (38%), at ground level (57%), at breast height (37%), in branches (38%), in twigs (100%), in leaf petioles (67%), and in leaf blades (8%) (Henry and Riker 1947).

MOVEMENT WITHIN THE HOST

Once inside red oaks, the pathogen spreads rapidly through the vascular system (Gregory 1971, Struckmeyer et al. 1958, Young 1949). Jacobi and MacDonald (1980) observed an initial slower colonization in red than in white (*Q. alba* L.) and chestnut (*Q. prinus* L.) oaks which may induce fewer early host responses and ultimately allow for greater colonization. Vessels of red oaks and white oaks, including those in leaf veins, are large enough to permit passage of conidia (2-3 X 5-7 μm). Some reports suggest that conidia are rare in xylem vessels (Parmeter et al. 1956, Struckmeyer et al. 1954, Wilson 1961). Fergus and Wharton (1957) did not observe fungal mycelia in twig and leaf petiole material, but tissue isolations on culture media from immediately adjacent areas often yielded pure cultures, suggesting that conidia or small hyphal fragments were present.

Conidia produced in vessels and tracheids are smaller than those produced in culture, averaging 1.2

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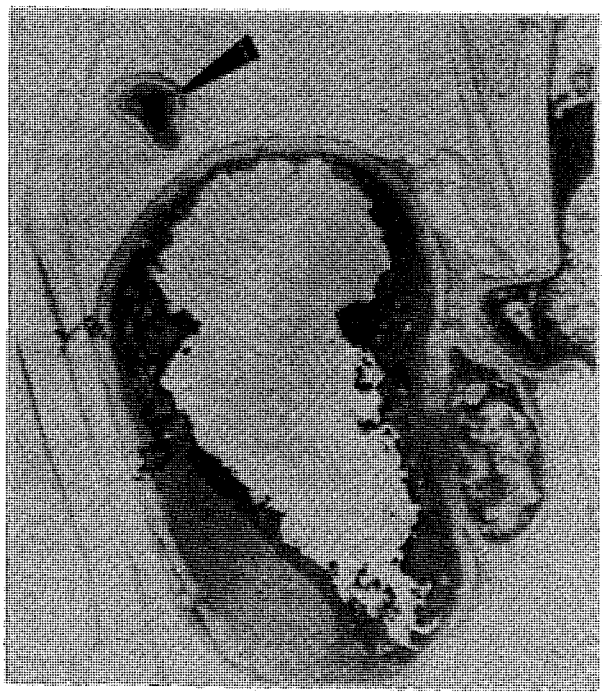
X 3.5 µm (Wilson 1961), and can easily be missed in the relatively small samples observed in histological studies. Although Struckmeyer et al. (1958) saw many spores and hyphae in vessels of inoculated live oak (*Q. virginiana* Mill.) seedlings, the general lack of hyphae in vessels until after wilting is advanced suggests that conidia are responsible for the pathogen's rapid internal distribution.

After wilting, extensive mycelial growth occurs in xylem vessels of the outer sapwood (Sachs et al. 1970, Struckmeyer et al. 1958). Hyphae then penetrate into the adjacent xylem parenchyma through the pits, later growing intercellularly and intracellularly within the cell wall. Cells through which hyphae have passed contain particles of dark-staining, granular material (Struckmeyer et al. 1958). Lysis of the infected cell wall proceeds from the lumen outward and eventually into the middle lamella (Sachs et al. 1970). Cavities of varying size, shape, and direction form within the cell wall, resembling the "soft rot" of sapwood commonly induced by Ascomycetes (Duncan 1960). Penetration through pits is more common than is direct penetration (Fergus and Wharton 1957) and the middle lamella is extensively invaded (Wilson 1961). Prior to 50% defoliation, hyphal invasion is extensive in ray and xylem parenchyma.

In artificially-inoculated northern red (*Q. rubra L.*) and black oak, time to first visible symptoms of wilting varies according to the point of inoculation (Henry and Riker 1947). For root and stem inoculations, the first symptoms develop on scattered branches in the upper crown. In trees which partially wilt and then recover, only to wilt in the spring of the following season, the fungus appears to migrate into the newly formed springwood (Struckmeyer et al. 1958). Wilt occurs only after actual fungus invasion of the current annual ring and plugging of the springwood vessels. *Ceratocystis fagacearum* also colonizes previous sapwood growth and is prevalent in the outer three rings shortly after appearance of wilt symptoms but may soon extend as deep as the tenth ring (Jones and Bretz 1955, Lewis 1987) if the tree is suitable for mat production. In Texas live oak (*Q. virginiana* var. *fusiformis* Sarg.), survival is greater in the root collar zone, presumably because of lower temperatures and higher moisture content (Lewis 1985c).

Figure 1. Portion of vessel element of *Q. alba*, showing tylose formation and gummosis. Note hypha (arrow) of *C. fagacearum*. (F.H. Tainter)



HOST REACTIONS

Living oaks require relatively large volumes of water for photosynthesis and transpiration. The large amount of water which evaporates from the leaves cools them and prevents heat damage to cells and tissues. Any stoppage in this flow of water will have rapid, lethal, and often dramatic results. Vascular wilt diseases such as that caused by *C. fagacearum* tend to be devastating in ring-porous trees like oak because water movement occurs in the current vessels. Exactly how vascular wilt pathogens initiate the wilting process is not well understood for most pathogens, including *C. fagacearum*. Large molecular weight-degraded cell wall components tend

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to become entrapped within the conductive elements (Hodgson et al. 1949) and reduce water flow through the system.

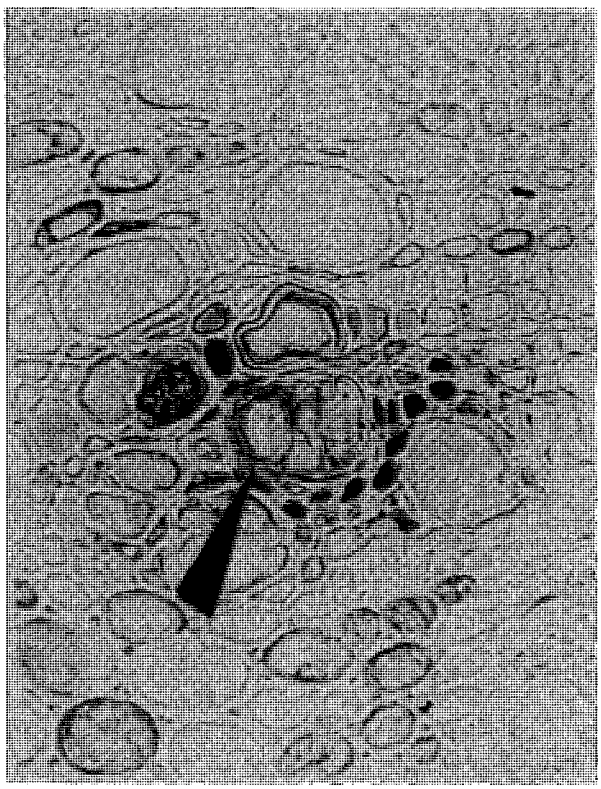
Smaller molecules move up the sapstream and accumulate in leaf margins, producing marginal drying (Hess and Strobel 1969). Pectolytic and cellulolytic enzymes may reduce transpirational flow (Dimond 1970). Mycelia or spores may partially fill the cross-sectional area of vessels which also may contribute an increased resistance to water flow. Another factor is the formation of air embolisms (Van Alfen 1989). Transpirational water is under extreme tension during hot, sunny days when transpiration is at a maximum. Fungus sporulation and growth may cause gas bubbles to form which quickly enlarge and block water flow.

The twigs in the upper crown of red oaks have larger vessel diameters than do lower twigs (Fritts 1976). This allows them to be more efficient for conduction of water into the crown, but the larger diameters are also at higher risk for formation of embolisms. This is one reason why the upper crown tends to wilt first. Toxins may affect the water-pumping ability of parenchyma cells. The manner of response in wilting suggests that *C. fagacearum* may produce a toxin(s) during early pathogenesis. It does produce phytotoxic substances in culture (McWain and Gregory 1972, White 1955).

The first visible external reaction to infection is the appearance of wilt symptoms in the leaves. Struckmeyer et al. (1954) noted severe plasmolysis of all cell protoplasts in wilted leaves. Wilting leaves are thinner than healthy ones and their plastids are not distinguishable. Leaf vascular tissues are shrunken but devoid of tyloses. Their petioles, however, are commonly occluded with tyloses and gums. In red oaks, visible symptoms begin with a slight crinkling and paling, often followed by a progressive bronzing to browning of the leaf blade from the lateral edges and apex toward the midrib and base (Henry et al. 1944). This often includes water soaking with a sharp line separating the discolored portions from the normal green basal part of the leaf (True et al. 1960).

Young leaves often droop conspicuously but mature leaves remain relatively rigid. Wilted young leaves may remain attached but defoliation of affected

Figure 2. Xylem of *Q. alba*, showing tylosed vessel element (arrow) surrounded by parenchyma cells filled with gum deposits.



mature leaves is more usual and may occur at any symptom stage (Henry et al. 1944). In infected white and bur oaks, leaves often become progressively necrotic from the apex toward the base, as they do for red oaks (Henry et al. 1944). Though sometimes bronzed, the discolored areas are often light tan to brown, or dark green, and somewhat watersoaked in appearance. Affected leaves tend to remain attached to the tree and to be scattered throughout the crown.

The foliar wilt symptoms in live oak vary but do not resemble symptoms on deciduous oaks. The most reliable symptoms in Texas live oak are veinal necrosis and tipburn (Appel and Maggio 1984). In deciduous red oaks, secondary foliage (Henry et al. 1944) and sucker shoots (True et al. 1960) may be

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abundantly produced on trunks and larger branches. These usually soon wilt and die. Their wilting pattern is distinctive, however, in that they blacken, curl, and wrinkle as though frost-injured, instead of turning bronze or brown (True et al. 1960).

In large artificially-inoculated red oaks, time from inoculation to appearance of first foliar symptoms ranges from 3 to 9 wk (Engelhard 1956). Ninety-six percent of black oak trees became symptomatic in the same season when inoculated, with the remaining 4% developing symptoms the following year (Jones 1964). One-hundred percent of trunk-inoculated northern pin oaks (*Q. ellipsoidalis* E. J. Hill) developed symptoms the same season, whereas only 40-80% of bur oaks did so (Nair and Kuntz 1963). In older seedlings or sprouts, the shortest incubation period was 14 dys. (Henry and Riker 1947), or 15-19 dys. (Gregory 1971). In 28-day-old northern red oak system development was from 6-11 dys. (Fenn et al. 1975). White oak seedlings consistently developed wilting symptoms more slowly (Fenn et al. 1975).

Plugging of vessels by amorphous materials (perhaps the earliest stage of gummosis) and tyloses is detectable as soon as four dys. after inoculation and at least two dys. prior to the first evidence of foliar symptoms (Fenn et al. 1975). Refer to Figure 1 which shows the vessel element portion of *Q. alba* with tylose formation and gummosis and also the hypha (arrow) of *C. fagacearum* (F. H. Tainter, unpublished). The plugging is viewed as electron dense material under the transmission electron microscope (Sachs et al. 1970). Tyloses in xylem vessels may occlude from 50-70% of vessels in the trunk (Jacobi and MacDonald 1980, Struckmeyer et al. 1958) to 100% of vessels in twigs (Beckman et al. 1953, Struckmeyer et al. 1954). The severity of wilt is dependent on the degree of tylose formation (Struckmeyer et al. 1954). Tyloses frequently form in sectors of the outer sapwood which are positionally related to the wilting branch (Struckmeyer et al. 1954). Tylose formation has been observed two to five dys. before wilting (Parmeter et al. 1956) and when resistance to water flow in the stem is greatly impeded (Gregory 1971).

TeBeest et al. (1976) found that stomatal resistance increased sharply from 7-10 dys. after inoculation in 28 day-old seedlings of northern red oak. Wilson (1971), however, has argued that death results from fungal invasion and killing of parenchyma tissues and not from lack of water caused by vessel occlusion. In oak-wilted trees, tyloses form as an extension of the simple pit membrane into the vessel lumen (Struckmeyer et al. 1954), between pit pairs of ray parenchyma cells and the vessels. Initially tyloses are small, forming along parts of the wall immediately adjacent to ray cells. As the tyloses come into contact with each other, the vessel becomes completely occluded. The tylose wall then becomes thickened and lignified. In red oaks, tyloses generally form only in the vessels of the last-formed ring but in some instances they also may form in the previous annual ring. Tylose formation in healthy white oaks is generally abundant, even in the outermost rings (Gerry 1914), and this may impede the spread of the fungus in these species. Roots of trees with severe wilt have tyloses but at low numbers (Struckmeyer et al. 1954) with the occluded vessels in sectors separated by multiseriate rays.

The earliest visible reaction within the sapwood of wilted oaks is brown to black vascular discolorations, a result of gummosis, usually in the most recent annual ring (Henry et al. 1944, True et al. 1960). Refer to Figure 2 which shows the xylem of *Q. alba* with the closed vessel element (arrow) surrounded by parenchyma cells filled with gum deposits (F. H. Tainter, unpublished). Gummosis increases during the later stages of wilt, especially in the small vessels of summerwood (Struckmeyer et al. 1954). Gums may also form in vascular rays, xylem parenchyma, and tracheids. They first appear as small droplets along the wall. These then enlarge and coalesce, filling the lumen. In cases of severe occlusion, tyloses and gums may be found together in the same vessels, appearing as diffuse, stippled, or continuous streaks of varying lengths. Discoloration is more common in the white oak group than in the red oak group (Henry et al. 1944, Parmeter, et al. 1956, True et al. 1960). Discoloration starts as orange-brown, then deepens into a dark brown (Parmeter et al. 1956). It is usually limited to the affected ring although the next inner ring occasionally also contains a small discolored area. No outward extension of the stain occurs beyond the infected ring.

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Discoloration is limited in the roots and is indistinct. In the southerly portion of the range of oak wilt, gummosis and tylosis is common in red oaks (Tainter and Fraedrich 1986, Tainter and Ham 1983).

MAT FORMATION

After the tree wilts and the cambium turns brown, mycelial mats and pressure pads may form. Their formation begins with fungus invasion of the outer sapwood, causing the proliferation of outer ray parenchyma cells near the cambium (Struckmeyer et al. 1958). Their subsequent disintegration facilitates the loosening of the bark and, in conjunction with pressure exerted by the enlarging fungus mat, promotes bark cracking. Curl (1955) described the mats and pressure pads as "a light to dark grayish tan, sparse to dense mycelial felt, in the center of which lies a thick gelatinous pad. The mycelial felt with its central pad are referred to together as a mycelial mat which varies in size from 1 x 1 cm to 48 x 14 cm.

The central pad is elongated oval to elliptical, dull green to black in color, and has a daedaloid surface. It is pseudoparenchymatous in structure and is usually free of visible loose mycelium." According to their stage of development mats are immature, mature, aging, declining, or deteriorating. Mats are initiated as hyphae growing between the cambium and the wood (Struckmeyer et al. 1958). The hyphae become organized into a pseudoparenchymatous stroma. Mats that become larger than 1 x 3 cm have a central core of hyphae surrounded by a mass of enlarged, rounded cells. Within this mass, 2 or more large cells become conspicuous and may form ascogonia. The mats and pressure pads, or cushions as they are also known, are usually associated with cracks in the bark and are recognized as a mechanism for liberating the spores (Leach et al. 1952).

As the fungus cushion expands, it pushes out on the bark and causes a longitudinal crack, beneath which forms a large moist chamber in which the sporulating mycelial mat develops. A mat measuring 24 x 10 cm was estimated to contain 1,650,000,000 conidia (Curl 1955). Perithecia (Barnett et al. 1952) also are produced on about 23% of the mats (Curl 1955). A distinctive nutty or fruity odor is associated with the mycelial mats and cushions, and is attractive to insects (Leach et al. 1952).

There is a "spotty" distribution of the fungus in the bole and larger branches during early stages of wilting (Boyce 1954, Boyce and Garren 1953, Henry and Riker 1947, Lewis 1985a, Nair and Kuntz 1963, Struckmeyer and Kuntz 1954, Tainter and Gubler 1973, Tainter and Ham 1983, Young 1949). Mat production seems to be dependent on an as yet unknown trigger as colonization of the sapwood reaches its maximum development. Mats are not formed immediately when a tree wilts. The cambium and phloem remain green and healthy-appearing for a time. Later they become light brown and the outer surface of the sapwood becomes water soaked (Campbell and French 1955b). Mat formation is dependent on complete wilting and subsequently occurs in just over four wk (Campbell and French 1955b) to four mo later (Fergus 1953). There is no relationship between size of the tree and mat formation (Campbell and French 1955b, Gillespie et al. 1957).

There is great variation in the number of mats formed on red oaks. On some trees no mats are formed. Conversely, one northern pin oak had 246 mats and pads (Engelhard 1955) and these were located from the base of the bole upwards and on branches as small as 2.5 cm in diameter. More than half of oak-wilted trees can be expected to produce mats either in the late summer or during the following spring (Gillespie et al. 1957). The later the occurrence of complete wilt, the longer is the duration of mat production (Campbell and French 1955b). In Arkansas and South Carolina, mats rarely form (Tainter and Gubler 1973, Tainter and Ham 1983). In Texas, mats are commonly formed on dead or dying Spanish oak (*Q. texana* Small) but not on Texas live oak (Appel 1986). Mats and pads may form on white oaks but at a much lower frequency (Engelhard 1955) and then only when the tree wilts very quickly. Larger mats are formed on the lower bole, but many smaller mats may be found almost to the top of the tree (Cones 1967).

Appearance of mats may be affected by several environmental variables. In Minnesota, mats are formed in May and June and from August through November (Campbell and French 1955b). No mats

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are found between December and April, presumably because the temperature is below freezing. In Iowa, mats are most abundant from August through October and from April through June but are found in every month (Engelhard 1955), even though they may become frozen in winter. In Pennsylvania, mats have been observed to form in March, May, June, August, September, October, and November (Fergus 1953). In the southern Appalachians, mats are produced in the summer or spring (Boyce 1954). In Arkansas, the few mats observed to form were found in November and December (Tainter and Gubler 1973). In Texas, mats first formed in November (Appel et al. 1987) and December, with peak production in March and then tapered off through April and May (Appel 1986). Curl (1955) reported that moderate temperatures and high sapwood moisture content are conducive for mat formation. Trees in which mats formed remained moist (Campbell and French 1955b, Fergus 1953). However, where mats did not form until the following year, the moisture content had dropped.

Various treatments aimed at reducing the number of mats have utilized early felling and deep girdling (Morris 1955). Felling diseased trees tends to induce mat formation in the fall, whereas the trees left standing produce mats the following spring and summer (Gillespie et al. 1957). Girdling of wilted trees in June and July is more effective in reducing moisture content than is girdling in late summer (True and Gillespie 1956). In both cases, the trees of small-diametered material dry more rapidly than do larger ones. Spilker and Young (1955) observed that as moisture content of stored inoculated wooden blocks decreased, the viability of the fungus in them also decreased. Boyce (1957) obtained a positive correlation between precipitation and frequency of mat formation on wilted trees, with a greater frequency of the trees producing mats during the wetter January-April period. For trees that began to produce mats during hot weather (Campbell and French 1955a), the period of mat production was of short duration. High temperatures decreased growth of the fungus or increased the rate of desiccation so that the sapwood became too dry for mat development.

In roots, the bark remains in contact with the cambium and there is no evidence of mat formation (Struckmeyer et al. 1958). Most radial growth of the fungus outward continues through the parenchyma cells. Some hyphae also penetrate cortical cells and the periderm.

DETERIORATION OF WILTED TREES

Within the first several minutes after spores of *C. fagacearum* enter the springwood vessels of susceptible oaks, a series of events is set into motion which makes the doomed tree more and more susceptible to attack by hordes of microorganisms and other biological scavengers. Dying oaks contain a rich assemblage of food resources for those organisms able to gain access and compete for it. The inner phloem and outer xylem have a high sugar content which, along with the high nitrogen content of the outer most growth rings (Merrill and Cowling 1966), make this zone especially attractive. The extant bark represents a rather formidable barrier but once it begins to crack and slough off, the relatively weaker cell types in the phloem/cambium zone facilitate mechanical and enzymatic entry by invaders.

The literature suggests that oak-wilted trees in the northerly portion of the range of oak wilt do not deteriorate as quickly as those growing in the southerly portion of its range, excluding that portion of its range in Texas. This may be due to more favorable temperatures and longer growing seasons, both factors of which would encourage competition for substrate. An early invader of wilted oaks in the Southeast is *Hypoxylon atropunctatum* (Tainter and Gubler 1973, Tainter and Ham 1983). This fungus is known to latently infect living bark tissues of oak seedlings from which it is able to rapidly colonize the host following stress (Bassett and Fenn 1984).

Growth of *H. atropunctatum* into living oak tissues is stimulated when their relative water content declines to 60-70% (Mason and Fenn 1990). This gives the fungus a positional advantage and it quickly colonizes the sapwood, metabolizes starches and sugars, and then produces a soft-rot of the cell walls (Tainter and Gubler 1974). Lewis (1985b) found *Hypoxylon* spp. and several other canker fungi associated with oak wilt-infected live oak and Texas

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red oak (*Q. texana*) in central Texas, as well as with trees expressing decline symptoms from other causes.

In West Virginia, *H. atropunctatum* has only been isolated from dead branches of wind-damaged trees or from non-girdled oak wilt trees (Shigo 1957). Perhaps because of lower mean temperatures, it is not as aggressive there, as it is favored by high temperatures (Davidson and Vaughn 1942, Thompson 1963), or it may not be able to colonize the living inner bark of oaks (Bassett and Fenn 1984). In West Virginia, *H. punctulatum* may be a more active colonizer (Amos 1965, Barnett 1957, Davis 1964, Fergus and Stambaugh 1957).

In Texas, *H. atropunctatum* colonizes oak-wilted trees (Appel et al. 1987), but does not seem to have a significant effect on reducing the incidence of oak wilt. Carbohydrate depletion by *Hypoxylon* spp. did not occur in Texas live oaks with oak wilt (Tainter and Lewis 1982). In the Piedmont of South Carolina, oak-wilted trees are exposed to an intense invasion by two-lined chestnut borers, *H. atropunctatum*, *Armillaria* spp., other decay fungi, termites, and woodpeckers (Tainter and McElreath 1992). Other related *Hypoxylon* species such as *Nummularia bulliardi* (= *Hypoxylon mediterraneum*) (Slabaugh 1971, Turk 1955), or other early invading fungi such as *Trichoderma viride* (Amos 1961), *Schizophyllum commune* (Slabaugh 1971, Turk 1955), and other woodrotting fungi (Brandt 1953, Henry et al. 1944) also may appear soon after the tree wilts and represent an early stage in the succession of organisms toward final deterioration.

CONCLUSIONS

Phenological and histological details of the interaction between *C. fagacearum* and some of its oak hosts provide a general view of what happens during and after infection. Many of the traditional, but essential, biological studies of the past decades have not been repeated with the species involved in the present epiphytotic in Texas. The relatively few studies there are relating to the biology of infection provide a tantalizing glimpse into understanding a fascinating disease occurrence which many early pathologists could not have predicted. There is a glaring deficiency in our basic understanding of the physiology and biochemistry of infection, regardless of region or species. Perhaps the current situation in Texas and the fear of introduction of *C. fagacearum* into European oak forests will stimulate research in this area. Until our understanding is more complete, it is not likely that control efforts will be as successful as we would like them to be.

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Modelling the Local Spread of Oak Wilt

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In stands of red oak species, the oak wilt fungus, *Ceratocystis fagacearum*, is disseminated through root grafts between trees. Pathogen spread models were developed by probit analysis of field data collected in Michigan. These models predict the probability of disease spread across root grafts to a healthy tree during the year after symptoms occur in a nearby diseased tree. Movement of the fungus was best predicted by the natural logarithm of the quotient obtained by dividing the sum of the diseased and healthy tree diameters by the distance between them. Multiple-stemmed trees were effectively represented by the sum of their stem diameters. Pathogen spread occurs over greater distances through deeper and sandier soils than through loamier soils. Tables were derived which provide the distance between a healthy and diseased tree corresponding to a 95 or 99 percent probability of healthy tree survival for a year. These tables have been used to objectively place barriers to root-graft spread of the oak wilt pathogen in Michigan.

INTRODUCTION

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, parasitizes and kills *Quercus* spp., especially members of the subgenus Erythrobalanus (red oaks). Stems and canopies of infected red oaks usually die within a few days to two weeks after symptoms appear. Though the root systems of infected trees may survive long enough to support stump sprout development, these sprouts often become infected and die within a few years. The oak wilt fungus spreads through root grafts between oaks and overland via insect vectors (Gibbs and French 1980, Juzwik 1983, Juzwik et al. 1985, MacDonald and Hindal 1981, True et al. 1960). An overview of Michigan's oak wilt management program is presented elsewhere (Bruhn, these preceedings). This paper presents a method for developing local models of root-graft spread of *C. fagacearum*. The model reported here predicts the probability of root-graft transmission during the year following appearance of symptoms in a diseased tree (potential source). We also demonstrate use of this model to produce reference tables.

In ten years of observation in pure stands of northern pin oak (*Q. ellipsoidalis* E. J. Hill) in northern Michigan, the only cases where oak wilt foci failed to advance into surrounding oak stands have been where barriers to root graft spread were successfully established. Reliable barrier placement depends on being able to identify all trees close enough to the advancing margin of the disease focus to become infected before barrier construction. An effective barrier must surround all such trees.

It has been estimated that more than 95 percent of the trees which died of oak wilt in Minnesota became infected via root-graft transmission (Juzwik 1983). We presume that rate of root-graft disease spread depends on the extent to which the oaks in a stand are connected by root grafts, and that this varies greatly with stand and site conditions. Disease centers in some areas may be limited to individual trees, whereas periods of one to several years, on average, may be required in other areas for root-graft spread of oak wilt (Gibbs and French 1980). Both

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Himelick and Fox (1961), working in southern Illinois, and Jones (1971), working in Pennsylvania and West Virginia, presented data which suggested that the maximum intertree distance over which root-graft spread might occur was at least 60 ft..

Correct placement of root-graft barriers is critical for efficient disease control. The cost of barrier construction is substantial in both direct expense and the sacrifice of healthy trees. The strategy of root-graft barrier placement is to contain the pathogen while leaving as few healthy trees as possible within the barrier. Barrier locations are determined between late July and mid-August, by which time most of the year's mortality has become evident. Since the exact position of the pathogen between diseased and healthy trees is uncertain, the attempt is made to include inside the root-graft barrier all apparently healthy oaks with a five percent or greater chance of becoming infected during the next year. The probability value (e.g., 1, 5, or 10 percent) should be selected on the basis of local tree values and cost of barrier establishment. The number of healthy oaks sacrificed to ensure containment of the pathogen can be large and increases with focus size.

In Menominee County, Michigan, barriers consist of vibratory plow lines cut with a five-foot long blade. The pathogen may avoid containment either by root grafts beneath the barrier or if the barrier is established within the perimeter of infected roots. The pathogen may also cross the barrier through root grafts formed subsequent to barrier establishment. Neither the rate of normal root graft formation between trees nor the rate at which root grafts form across vibratory plow lines has been documented. Nevertheless, a model which effectively predicts the probability of disease spread between two trees under local conditions has been extremely useful for properly placing root-graft barriers.

METHODS AND MATERIALS

Site Description

The data used for model development were collected from 49 disease foci in Menominee County, Michigan. The area of concern was 10,000 acres of well-stocked red oak forest, lying in 48 contiguous sections of land. The predominant species in these stands was northern pin oak. The foci studied were located in mapping units of the Grayling and Pemene soil series. The Grayling series is a deep, excessively drained, sandy deposit on outwash plains and lake plains, typically 15-30 in. deep. The Pemene series is a deep, well drained, loamy sand glacial till found on ground or end moraines, typically 24-48 in. deep (National Cooperative Soil Survey 1982, 1985).

Data Collection

Oak wilt was introduced into Menominee County, MI, by the early 1950s. Since 1981, disease progress has been documented in each known focus from the date of diagnosis. The locations, diameters, and disease status of trees were monitored. Diagnosis was based on foliar and vascular symptoms, proximity to trees with mats, and production of mats, and/or isolation of the pathogen in pure culture.

The extent of overland disease spread within the foci has not been studied *per se*, but is apparently very slight. Unlike the situations reported by True et al. (1960) and Jones (1971), in Menominee County there is a definite break in the frequency distribution of observed intertree disease spread distances which permits clear distinction between root-graft disease spread as opposed to overland spread which establishes new foci. We have not seen evidence of oak wilt spread over intertree distances greater than 60 ft in the vicinity of any of our foci.

Each diseased tree was considered a source of inoculum for potential root-graft spread. From each source tree, all possible paths of root-graft disease spread were identified. Each path was represented for modelling purposes as the straight line vector connecting a potential source tree to a surrounding tree which was healthy at the time of source tree death.

Well over 4,000 trees have died of oak wilt in the study area to date. The path of root-graft transmission was not always clear, due both to timber/firewood harvests prior to 1982 and to unsuccessful attempts at chemical control. Cases where potential source trees at the margin of a focus had been killed more than one year prior to barrier placement also were excluded from use in model

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development. Thus, the total dataset representing single-year root-graft pathogen spread consisted of 655 observations: 522 cases involved one single-stemmed diseased tree and one single-stemmed healthy tree (evaluation of independent variables during initial model development was based solely on these cases); 60 cases involved multiple-stemmed trees for either the potential source or target tree; 73 observations involved more than one possible source tree.

Preliminary Model Selection

A mortality model should provide estimated probabilities bounded by zero and one (Hamilton and Edwards 1976). A sigmoid cumulative probability distribution such as the normal density is biologically reasonable. At each end of the probability distribution, there is a range where a specific change in the independent variables has little impact on the predicted probability. However, in the middle of the distribution, the same change in the independent variables will cause a much larger change in predicted probability. As an example, the addition of 10-ft distance for a susceptible healthy tree will have almost no effect on the chance of disease transmission if the target tree is already 100 ft away. However, if the target tree is 40 ft away rather than 30 ft, a significant change in the probability of disease transmission can occur.

For this study, probit analysis (which utilizes the normal probability density function) was used to predict the probability of oak wilt root-graft spread between trees within a single year. The generalized probit model was

$P_i = F(b'x_i)$, where:

- P_i = annual probability of disease spread for the ith tree,
- F = a cumulative probability function,
- b' = a vector of estimated parameters, and
- x_i = a vector of predictor variables for the ith tree.

In binary choice models, the two possible results are assigned values of one or zero. With oak wilt, successful transmission of the disease was assigned a value of one and failure of disease transmission a value of zero. Parameter estimation for this project utilized the RATS software (Regression Analysis of Time Series, VAR Econometrics, Minneapolis, MN, 1986).

Nine models were selected for analysis (Bruhn et al. 1991). Models tested were selected to; 1) keep the model as simple as practical, and 2) include combinations of independent variables which made biological sense. The source tree and target tree diameters were included either as separate independent variables or summed, which weighted both diameters equally. Intertree distance also was used as a separate independent variable. Summed diameters were used as the numerator in several ratio-type variables, with intertree distance as the denominator. This functional form of model was biologically appealing because all three predictor variables were simultaneously included. The model formulations that used the variables independently implicitly assumed that the variables did not interact.

Preliminary testing indicated that, when the ratio of summed diameters to intertree distance was the only independent variable, the resulting model displayed a pronounced lack of fit. For intermediate probabilities of death, far more trees were predicted to die than actually died. Furthermore, very few observations resulted in low probabilities of death. This type of lack of fit is often addressed with a nonlinear transformation of the independent variable. Consequently, the natural logarithm of the ratio variable was used as the independent variable.

Testing of Qualitative Variables

The influences of; 1) the two soil series, 2) the year of data collection, and 3) trees with multiple stems were tested to determine if any additional variation could be explained. Each of these issues was addressed through the use of classification (dummy) variables (Searle 1971), with a t-statistic calculated to determine if the coefficient of the classification variable was significant.

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RESULTS

Data Analysis

Probit regression analyses using RATS were conducted for the nine selected model forms. Only single-stemmed target and source trees, and only target trees with one likely source tree, were included in these initial analyses. The resulting models were evaluated using the chi-squared goodness-of-fit statistic (Ostle 1963). Three of the nine models were eliminated at this step due to lack of fit.

Next, a truth table was constructed for each model to compare the number of cases of disease transmission predicted with the actual field outcome. This provided a simple method for comparison of model performance. At this point, the models with the poorest truth table performance were eliminated. Models 1 and 2 (see below) provide similar results. Model 2 provided uniformly superior performance in the truth table, but had a functional form more complicated than Model 1. Both models were retained for further analysis. Two other models were eliminated because they were similar to, but more complex than, Model 3, and neither performed better than Model 3 in the truth table evaluation. Model 3 was retained for further analysis.

Final Model Selection

The following three models provided acceptable results, based on chi-squared and truth table analyses.

$$y = b0 + b1*dtt + b2*dst + b3*D \quad (1)$$

$$y = b0 + b1*dtt + b2*dst + b3*\ln(dtt) + b4*\ln(dst) + b5*D \quad (2)$$

$$y = b0 + b1*\ln((dtt+dst)/D) \quad (3)$$

In these models, y was the Z score associated with the annual probability of disease spread, dtt was the dbh of the potential target tree, dst was the dbh of the source tree, D was the distance between the target and source trees, b0 was the y-intercept, and b1 through b5 were coefficients for the independent variables.

For each of the three remaining models, the critical distances between target and source trees were calculated for a range of target and source tree diameters. The critical distance was defined as the distance corresponding to a 95 % probability that the target tree would survive for another year following symptom appearance in the source tree. Model 3, which gave the greatest range of critical distances (4 to 92 ft), was felt to be the most biologically sound, and was selected for further testing.

An additional goodness-of-fit chi-squared statistic was calculated for Model 3 to evaluate the fit of our selected model (Bruhn et al. 1991). A data distribution table was established in which the number of trees observed to have lived or died and predicted to have lived or died were categorized into 10 ft intervals of intertree distance and 4 in. classes of summed diameter. The calculated chi-squared statistic for this distribution of the data was 72.502 ($k = 0.05$, df=121), indicating that the model predicted mortality well over the range of the data.

Testing of Model Variables

Soil type was a significant predictor of mortality ($k = 0.005$). Critical intertree distances were approximately 20 % shorter for soils mapped to the Pemene series than for those mapped to the Grayling series (Table 1). As depth of rooting area is reduced when all other variables are held constant, lateral root extension and subsequent disease spread might be expected to increase (Gillespie and True 1959). By definition, soils of the Pemene series average up to 18 in deeper than soils of the Grayling series. Also, the bands of loamy texture which characterize Pemene soils are expected to reduce lateral root extension.

The effect of multiple-stemmed vs. single-stemmed trees did not prove to be significant. This result facilitated model application; any tree could be effectively represented by a single diameter value, regardless of its number of stems.

Final Model Adjustment

Initial models included an intercept term. However, the constant did not prove to be significant for the chosen model, and removing it resulted in an

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Table 1. Intertree distances (in ft) associated with 95% and 99% probabilities, on soils of either the Pemene or Grayling Series, that a living tree will not contract oak wilt through root graft spread from an inoculum source tree within a one year period following its death, based on the combined diameter (in inches) of the two trees.[1]

	Intertree Distance (ft)			
	95% Confidence Interval		99% Confidence Interval	
Combined [2] (in.)	Pemene soil	Grayling soil	Pemene soil	Grayling soil
2	3.1	3.9	4.1	5.1
4	6.2	7.8	8.1	10.2
6	9.3	11.6	12.2	15.3
8	12.4	15.5	16.3	20.4
10	15.4	19.4	20.3	25.5
12	18.5	23.3	24.4	30.6
14	21.6	27.2	28.5	35.7
16	24.7	31.0	32.5	40.8
18	27.8	34.9	36.6	46.0
20	30.9	38.8	40.6	51.1
22	34.0	42.7	44.7	56.2
24	37.1	46.6	48.8	61.3
26	40.2	50.4	52.8	66.4
28	43.2	54.3	56.9	71.5
30	46.3	58.2	61.0	76.6
32	49.4	62.1	65.0	81.7
34	52.5	66.0	69.1	86.8
36	55.6	69.8	73.2	91.9
38	58.7	73.7	77.2	97.0
40	61.8	77.6	81.3	102.1
42	64.9	81.5	85.4	107.2
44	68.0	85.4	89.4	112.3
46	71.1	89.3	93.5	117.4
48	74.1	93.1	97.5	122.5

[1] The equation $D = (dtt + dst)/e^{([-Z - (-0.5659735 * (soil))]/2.481853)}$ was used, where: D = intertree distance, in ft; dtt = healthy target tree in inches; dst = potential inoculum source tree in inches; e is the base of natural logarithms; soil = 1 for the Pemene Series or 0 for the Grayling Series; Z = 1.645 for the 95% confidence interval, and Z = 2.33 for the 99% confidence interval.

[2] dtt + dst.

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increased number of correctly predicted cases. The modified model was:

$y = -0.56*S + 2.48*\ln[(dtt+dst)/D]$, where;

S = I for a Pemene soil, or
S = 0 for a Grayling soil.

As discussed earlier, it is desirable to construct two barriers. For this reason, tables providing distances based on different probability levels for target tree survival were derived. The inner barrier encloses all apparently healthy trees with less than a 95 % probability of surviving one year, while the outer barrier encloses additional apparently healthy trees with probabilities of surviving between 95 and 99 %.

DISCUSSION

Although it is clearly preferable to know the soil series and use the appropriate mortality model, it is often inconvenient to determine the soil series associated with specific foci. To expedite barrier placement, tables developed from the Grayling soilmodel have been used since 1987 to position root-graft barriers. The Grayling model will be conservative (leaving more healthy trees within the inner barrier than necessary) on Pemene soils, but the Pemene model will often fail to contain the pathogen on a Grayling soil. Use of either model outside the area from which our data were collected may be risky. However, our Grayling series model might prove useful in other areas until local models can be developed.

Using the summed diameters of an infected (source) tree and a nearby apparently healthy (target) tree, Table 1 provides the critical distance by which these two trees must be separated for the target tree to have at least a 95 % or a 99 % chance of surviving one more year following symptom development in the source tree, on a Pemene or Grayling soil. A sprout clump is represented as the sum of the diameters of its stems. If two trees are closer than the 95 % value, then the inner barrier should be placed outside the apparently healthy tree. Our experience with the model is leading us to place increased confidence in the effectiveness of the 95 % barrier. Figure 1 presents an application of Table 1, for comparison of proper placement of barriers on both soils. Note that single year disease spread is not necessarily confined to the first ring of apparently healthy oaks. In fact, assignment to the first ring of trees is quite arbitrary.

A 95% barrier is defined as a barrier which surrounds all trees with individual probabilities lower than 95% of surviving for at least one year after symptoms appear in a potential inoculum source tree. As a result, in stands where varies, a 95% barrier will also surround some healthy trees with probabilities greater than 95% of surviving for one year. Thus, disease does not always spread in a direct progression from tree to tree, as small diameter trees may be temporarily bypassed as the disease spreads first to a larger tree somewhat farther from the inoculum source tree. Also, many trees immediately outside the inner barrier have probabilities greater than 95% of surviving for another year. Thus, less than five percent of these trees are expected to become diseased during the subsequent year, even without barrier establishment. This percentage is further reduced by establishing barriers early in the autumn after symptoms appear, because the model assumes that the pathogen will have up to a year to reach a target tree and evoke symptoms.

The actual probability that a specific barrier will contain an entire oak wilt focus is affected by; 1) the probabilities of one-year survival for all of the potential target trees left outside the barrier, 2) the elapsed time between symptom production and barrier construction, and 3) the exact location of the barrier between inoculum source and target trees. Thus, it is not possible to use the model to estimate the actual probabilities of disease containment achieved by barriers.

We believe that, prior to model development, our desire to save trees led us to locate many barriers too close to diseased trees to contain all infected roots. As a result, our barriers averaged much less than 95% probability of disease containment. The result of such errors was "failure to contain" the pathogen, though this was usually misclassified as "escape."

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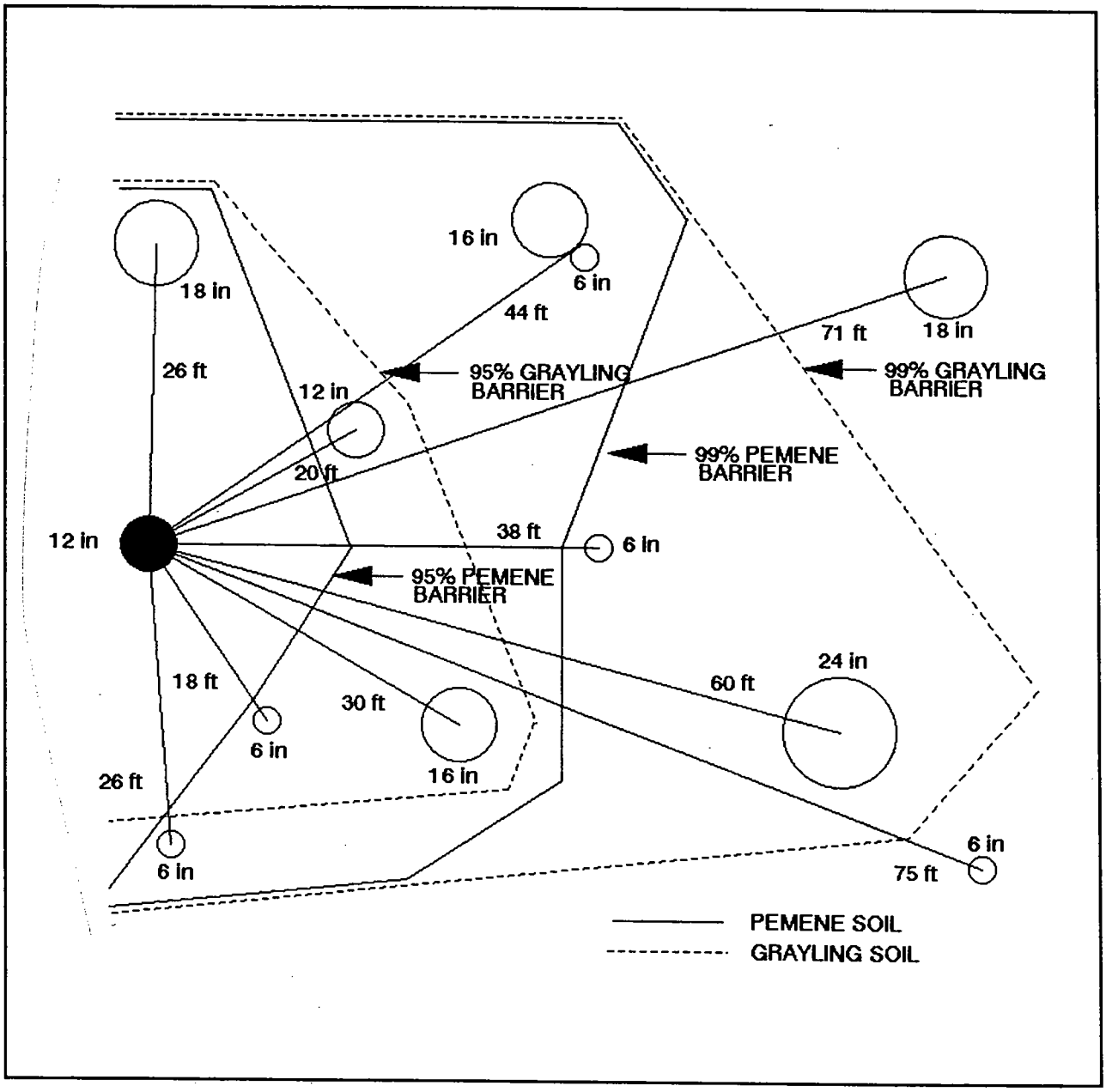
Figure 1. Proper location of root-graft disease spread barriers in Greyling and Pemene soils in Meniminee County, MI. The shaded circle represents an infected red oak; the open circles represent apparently healthy oaks.



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Wherever relatively homogeneous stands exist on relatively homogeneous soils, the modelling approach presented here will afford uniformly unbiased placement of barriers.

Use of Table 1 outside of Menominee County, MI, is risky because the epidemiology of oak wilt spread through root grafts is affected by soil, host, and stand factors. The extent of root grafting among oaks in a stand depends on the degree of species mixture, the propensities of the oak species present for inter- and intraspecific grafting, and local soil and drainage characteristics. Nevertheless, because the basic mechanism of root-graft spread remains the same, the procedures for modelling spread presented here are applicable in other areas.

ACKNOWLEDGEMENTS

Adapted from Forest Science 37: 28-44, published by the Society of American Foresters, 5400 Grosvenor Lane, Bethesda, MD 20814-2198. Not for further reproduction.

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The Threat of Oak Wilt to Europe

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Oak wilt, caused by *Ceratocystis fagacearum*, is presently found only in the midwestern and eastern United States. Its spread in most areas on native oak species has been slow and sporadic. The threat that oak wilt poses to the commercially important oak forests of Europe has been reinforced by the recent reintroduction of the Dutch elm disease pathogen, *Ophiostoma nouveau-ulmi*, into Great Britain. This concern has been manifested in various quarantine measures which have caused hardships in the worldwide trade of oak logs and lumber. A cooperative project among the European Economic Community, Clemson University, and West Virginia University has been underway for the past decade to examine the susceptibility of European oaks to oak wilt. This article examines some of the details of research methodology that must be clarified before major inoculation tests are conducted in the mid-1990s.

INTRODUCTION

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has been considered an internationally important disease since its discovery in the 1940s because the danger of this pathogen to oak species on other continents is unknown (USDA Forest Service 1963). As a result, quarantine regulations were established soon after the discovery of the disease that restricted the export of oak lumber and logs to Asian and European countries (USDA Forest Service 1978). These regulations were strengthened in the late 1970s following European experience with the reintroduction of a more aggressive strain of the Dutch elm disease (DED) fungus *Ophiostoma nouveau-ulmi* (Brasier 1979). This event led to significant elm losses in Europe and served to remind everyone of the hazards of shipping material such as logs that harbor dangerous pathogens.

Because the fungi that cause oak wilt and DED are vascular pathogens that are closely related, the assumption was made that oak wilt posed the same threat as DED. However, the spread of oak wilt in the United States has been slow and sporadic and a relatively low number of infected trees exist. Furthermore, oak logs and lumber have been exported to Europe for more than two centuries without any evidence that the disease has ever occurred on the European continent. European scientists have accepted the premise that the risk of exporting the pathogen was small, but they did not and should not accept any argument that experience with oak wilt in the United States reflects its potential impact to European oak forests. The forests of Europe are populated by different oak species that may differ greatly in their susceptibility to the oak wilt fungus. Furthermore, other insects, including the European oak bark beetle (*Scolytus intricatus* Ratzeburg), may be more effective vectors of the pathogen than are any known U.S. vectors. Therefore, additional safeguards were sought to further minimize the risk of international movement of the fungus.

OAK EXPORT SAFEGUARDS OF THE EUROPEAN OAK RESOURCE

Development of Current Export Regulations

The escalating concern over the risk of introducing *C. fagacearum* into Europe resulted in the passage of the

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European Economic Community (EEC) Plant Protection Directive 77/93. This 1977 EEC directive dealt with the importation of oak materials to member nations. In an effort to minimize the risk of introducing *C. fagacearum*, the directive required the removal of all bark and either; a) complete sapwood removal, b) a moisture content less than 20% or, c) disinfection through hot air or water treatment. Previous to this directive, oak logs were shipped carrying a phytosanitary certificate issued by the USDA Animal and Plant Health Inspection Service (APHIS) indicating that trees were from disease-free counties. Upon learning of Directive 77/93, most oak exporters and importers predicted that the new regulations would significantly reduce U.S. lumber exports and totally eliminate the export of oak logs.

Through negotiations with the EEC, officials of the U.S. government and industry, as well as university representatives, were successful in delaying implementation of the directive (Jakes 1992). The delay was granted after it was argued that time was needed to find procedures and/or establish systems that not only significantly reduce the risk of introducing the oak wilt fungus into Europe but also maintain trade. Several delays actually were necessary to resolve the issue. Initially, the EEC requested that the United States. formulate a new plan to certify the county origin of logs to be exported. The estimated cost of resurveying for oak wilt and implementing a log certification system that ensured that logs came from disease-free counties made this request unrealistic.

During this same period of time, research was initiated to develop new certification procedures. Studies were conducted to evaluate the efficacy of methyl bromide fumigation to kill the oak wilt fungus in logs and lumber. Other tests were designed to measure fungus survival in air-dried lumber. This research was funded by The National Lumber Exporters Association with the USDA Forest Service acting as the coordinating government agency. Unfortunately, air drying lumber required unacceptably long time periods to kill the fungus (Tainter et al. 1984), but methyl bromide fumigation was completely effective in eradicating *C. fagacearum* from logs without product degradation (MacDonald et al. 1985). As a result of this experimentation, EEC Directive 77/93 was amended in 1983 to permit log fumigation. Current EEC export requirements generally are as follows:

- Oak lumber (all species) - Boards must be stripped of all bark and edged, or treated by kiln drying, hot air, or hot water.

- Oak logs (white oak: Oct 15 - April 30) Shipped freely after chemical color tests to confirm species identification.

- Oak logs (red and white oak: May 1 - Oct 14) Fumigation treatment (T-312) with methyl bromide required.

(The above conditions are certified by APHIS following guidelines specified in the EEC Export Certification Manual. Some EEC member nations prohibit the importation of any oak logs with bark.)

The European Oak Resource

Twenty-three percent of the countries that comprise the EEC are forested, with oak being the most common timber species. More than half the oak produced in Europe comes from France, making it Europe's leading producer and second only to the United States in world production. Unlike the oak diversity that exists in North America, oak is represented by a small number of species that all belong to the subgenus Lepidobalanus. The most valuable species for timber production and the most common oaks are *Q. robur* L. (= *pedunculata*) and *Q. petrae* (Matt.) Liebl. (= *sessiliflora*), respectively. These two species belong to the Robur section and are well adapted to an oceanic climate or, in the case of *Q. pedunculata*, to a continental climate. *Quercus pubescens* Willd. also is in the same section, and its range is partly mediterranean. Several other evergreen species, including *Q. suber* L. and *Q. ilex L.*, grow in the Mediterranean Region but belong to other sections.

North American northern red oak (*Q. rubra* L.) was introduced to Europe near the end of the 17th century. At that time it was considered an amenity tree, but because of its rapid growth, foresters have

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begun paying considerable attention to the species. In recent years the tree has been planted widely so that many young red oak plantations currently exist. For example, in 1986-87, 1.6 million red oaks were planted in France; this represented 10 percent of the hardwood plantations established. Thus, the widespread occurrence of native and introduced oaks in Europe and their increased use have given rise to an increased concern or appreciation of their susceptibility to oak wilt.

One shortcoming in assessing the threat of oak wilt to Europe was the lack of information on the relative susceptibility of European oaks to the disease. Only one previous study examined the behavior of European oaks when inoculated with *C. fagacearum* (Bretz 1955). In this test, 1-2 yr-old seedlings of *Q. haas* (related to *Q. robur*), *Q. ilex*, *Q. macrolepis*, *Q. robur*, and *Q. thomasii* were inoculated and became symptomatic after 3-5 weeks. Unfortunately, subsequent disease development and host reaction were not described. The lack of knowledge about susceptibility was acutely recognized by the European and U.S. scientists who were dealing with the export/import issue. Their concern about oak wilt resulted in the EEC establishing an oak wilt research fund. One of the funded research projects was to study the susceptibility of European oaks to *C. fagacearum*. Obviously such research had to be done in the United States and not in Europe. The findings described in this paper are preliminary to a large-scale European oak susceptibility test that will be conducted in the mid-1990s with trees that will be approximately 10 years old.

ESTABLISHMENT OF EUROPEAN OAK ARBORETA

The EEC susceptibility trials began in the summer of 1980 when John Gibbs (Great Britain) and Jean Pinon (France) representing the EEC approached William MacDonald and Frank Tainter about the possibility of screening provenances of European oaks for their relative susceptibility to *C. fagacearum*. Two sites were desired. West Virginia was chosen to test northern provenances and South Carolina was chosen to test the southern provenance. The screening was to be done under field conditions with larger trees rather than young seedlings. This meant that 10 or more years were needed to establish test plantings from seed imported from Europe.

Eventually it was decided to maintain a single small arboretum on university properties in each of the two states. These could be established and maintained with the least expense and were under some degree of control. The EEC was to provide all the acorns. Acorns representing 11 and 14 provenances were supplied for planting in West Virginia and in South Carolina, respectively (Table 1). These were stratified when necessary and planted in nursery beds and held there through one or two growing seasons. Several shipments of acorns were made during the following 2-3 years. Many problems were encountered in the nursery and with establishment of the respective arboreta. Viability of acorns of some provenances was very low when they arrived. With other provenances, germination was good but survival of seedlings in the nursery was low.

Conversely, some species such as *Q. pedunculata* and *Q. sessiliflora* have grown quite well, much better than even the native white and red oaks planted as controls. This behavior has been noted by others (Clausen 1983). Predation by squirrels was a problem and although beds were covered with hardware cloth, there were losses. In West Virginia, browsing by deer necessitated that the arboretum be enclosed with a barbed wire fence. In South Carolina the decade of the 1980s was much hotter and drier than usual and drought-related losses after outplanting were probably much greater than they might have been if weather conditions had been normal.

Initially, plantings in South Carolina were on a 6 X 6-ft spacing with the intent of a total of two sets of 50 trees per provenance. Locations of provenances were placed randomly within the grid. In West Virginia, the planting consisted of 25 rows with 27 trees in each row (2 each of 13 provenances and 1 of PMN; see Table 1 for species and provenance labels). As losses occurred in South Carolina, the double sets of 50 were necessarily adjusted to one intended set rather than two. Extra seedlings were maintained in the nursery to replace trees which died. European oak trees that survived the first several years after

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Table 1. Statistics of oaks used in the cooperative oak wilt susceptibility study.

Species	Provenance	Year Planted	Label	Survivors as of 5/92
	West Virginia			
Quercus pedunculata	Germany	1984	DRF	50
Q. pedunculata	France	1984	NBR	50
Q. pedunculata	France	1984	LSN	50
Q. pedunculata	Italy	1984	PMN	25
Q. pedunculata	France	1985	FRX	50
Q. sessiliflora	France	1984	NRF	50
Q. sessiliflora	France	1984	LNX	49
Q. hybrid	Netherlands	1984	DWG	50
Q. hybrid	France	1984	SDZ	50
Q. petrae	Scotland	1986	GLN	48
Q. petrae	Scotland	1986	CSC	50
Q. rubra	United States	1984	ARO	50
Q. alba	United States	1984	AWO	47
Q. prinus	United States	1984	ACO	43
	South Carolina			
Quercus pedunculata	Germany	1984	MNC	36
Q. pedunculata	France	1984	TRB	27
Q. pedunculata	France	1984	NBR	35
Q. pedunculata	Great Britain	1985	WST	31
Q. peduncluata	Great Britain	1985	BRM	38
Q. sessiliflora	Germany	1984	WRN	8
Q. sessiliflora	France	1984	BRC	12
Q. sessiliflora	France	1984	NRF	15
Q. hybrid		1984	VLA	2
Q. ilex	France	1984	ITL	7
		1986		
		1989		
Q. petrae	Scotland	1986	GNT	9
Q. petrae	Scotland	1986	GLN	62
		1988		
Q. pubescens	Italy	1984	FLR	25
Q. suber	France	1984	STL	37
		1986		
		1988		
Q. rubra	United States	1984	RUB	36
Q. alba	United States	1984	ALB	41

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outplanting have generally grown well. Their American counterparts have generally grown less vigorously and there is some concern that they may not be large enough for the large-scale inoculations planned in the mid-1990s.

ESTABLISHMENT OF EEC SUSCEPTIBILITY TRIALS

Preliminary Evaluation of Seedlings

Although the goal of this cooperative project was to evaluate the response of European oaks to infection by *C. fagacearum*, details of methodology had to be worked out to ensure that the final screening would be as realistic and as useful as possible. It was decided that extra seedlings could be effectively utilized in various tests that would provide preliminary information to help answer questions regarding some of these details. Specific issues to be addressed included; 1) inoculum (isolate) source(s) of *C. fagacearum* to be used, 2) time of inoculation for optimum symptom development, 3) location or site on tree where inoculations would be made, and 4) development of a wilt screening estimation system that would detect possible differences in susceptibility among the species and many provenances.

Young Seedling Inoculations

This test was conducted in 1984 and used extra 1- or 2-yr-old seedlings of *Q. ilex*, *Q. suber*, *Q. sessiliflora*, *Q. alba*, *Q. pedunculata*, and *Q. coccinea*. Seedlings were inoculated at the base of the stem with either 1 X 10^4 spores/ml of a South Carolina (SC-2) isolate of *C. fagacearum* or with sterile distilled water. Twig tips, upper and lower stems, and roots were sampled at 3, 28, 53, or 76 dys., cultured for recovery of *C. fagacearum*, and histological samples were taken for examination with light and electron microscopy.

Seedlings of all European oaks had some wilt by day 76 but remained alive. *Ceratocystis fagacearum* was recovered from 31% of twigs, 10% of upper stems, 38% of lower stems, and 21% of roots. A compartmentalization reaction was evident at day 28 and after at all four sample sites. This reaction was visible through the light microscope as densely stained vertical strands of xylem with some tyloses present in all four sample locations. Hyphae of *C. fagacearum* were sparse but were observed in fibers and in vessels. Hyphae often appeared moribund. One conclusion of that limited study was that the European provenances were susceptible to *C. fagacearum* but that their reactions to infection appeared to be more similar to that of American white oaks rather than of American red oaks (Tainter et al. 1986).

Seasonal Susceptibility Test

A seasonal susceptibility test was conducted in 1986 to determine when during the growing season and at what location on the tree inoculations should be made for maximum effectiveness. Results of the South Carolina test were quite variable but the earlier date (May 6) resulted in quicker symptom expression and a somewhat more rapid increase in the percent of seedlings of European provenances exhibiting symptoms (Figures 1 and 2). Unfortunately, there were insufficient seedlings of American provenances to inoculate at the later dates. The May 6 inoculations resulted in 60% incidence of infected seedlings for stem-inoculated RUB and 0% for stem-inoculated ALB (see Table 1 for species labels).

In general, stem inoculations produced a greater incidence of wilt symptoms than did branch inoculations for a given provenance; this was true for both inoculation dates. Interestingly, wilt progression did not advance to include the total crown except for RUB which, incidentally, had relatively few leaves in comparison with the very leafy European provenances. The development of symptoms from the early inoculations was much easier to see and quantify. The June 5 and especially the August 18 inoculations were so late in the season that insect and powdery mildew attack and general leaf senescence made wilt symptoms very difficult to detect and quantify.

In the West Virginia seasonal susceptibility test, somewhat different results were observed. Since the growing season began much later, the first inoculations were done on June 19 (Figure 3). Those inoculations produced wilt symptoms in 100% of the seedlings of NBR and ARO and in 60-80% of NRF,

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Figure 1. Percent of European and American oak seedlings with wilt growing in South Carolina and inoculated with 1.1 X 10^6 spores/ml of a South Carolina isolate (SC-2) of *C. fagacearum* on May 6, 1986, at either of two inoculation sites.

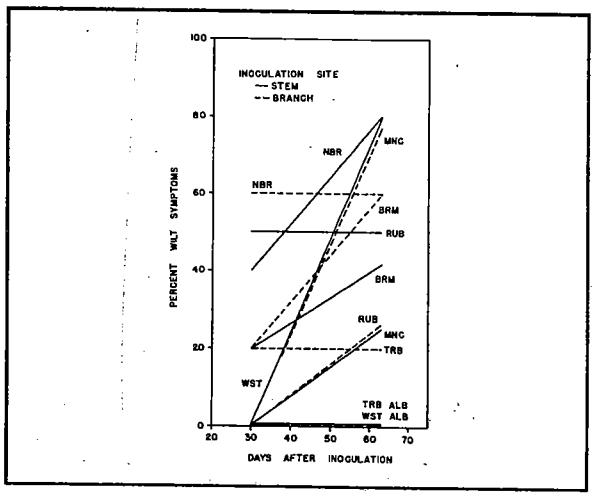

Figure 2. Percent of European and American oak seedlings with wilt growing in South Carolina and inoculated with 1.2 X 10^6 spores/ml of a South Carolina isolate (SC-2) of *C. fagacearum* on June 5, 1986, at either of two inoculation sites.

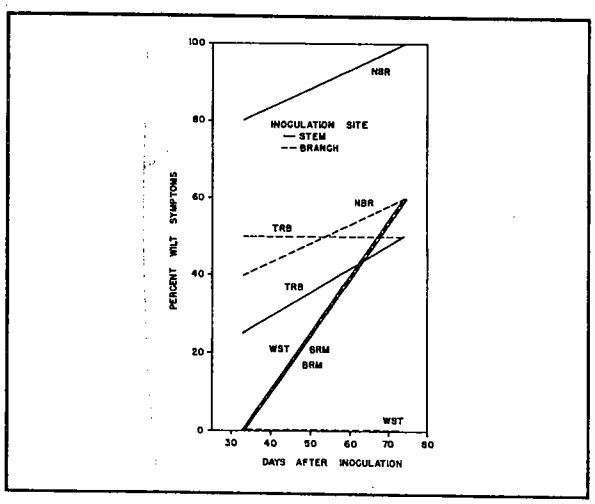

Figure 3. Percent of European and American oak seedlings with wilt growing in West Virginia and inoculated with 9.5 X 10^5 spores/ml of *C. fagacearum* on June 9, 1986, at either of two inoculation sites.

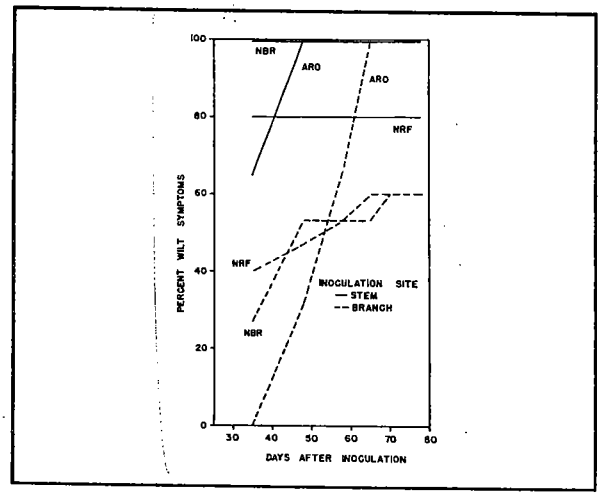

Figure 4. Percent of European and American oak seedlings with wilt growing in West Virginia and inoculated with 10.2 x 10^5 spores/ml of *C. fagacearum* on July 24, 1986, at either of two inoculation sites.

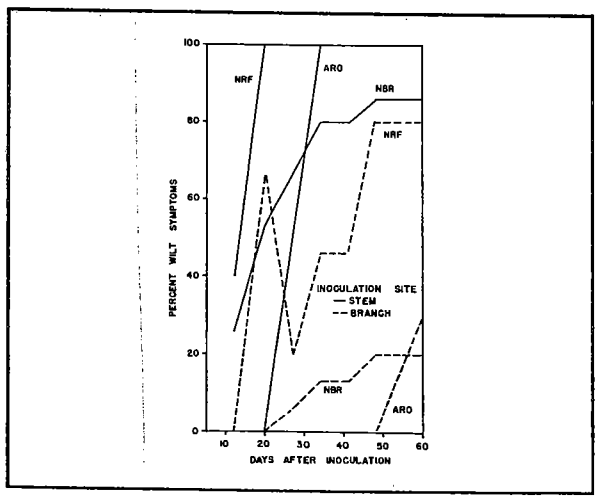

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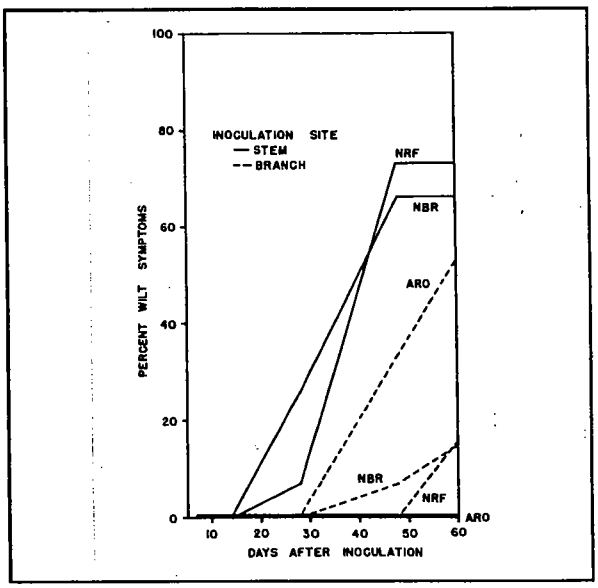
Figure 5. Percent of European and American oak seedlings with wilt growing in West Virginia and inoculated with 5.0 x 10^5 spores/ml of *C. fagacearum* on August 22, 1986, at either of two inoculation sites.



depending on whether the inoculation site was the stem or a branch. Trees receiving branch inoculations were generally slower to produce symptoms than were those with stem inoculations. Inoculations on July 24 produced somewhat more rapid wilt in the stem inoculated seedlings, but in NBR and ARO, wilt expression was slow to develop (Figure 4). The August 22 inoculations of stems produced wilt symptoms in fewer than 80% of the seedlings of the most affected provenances (NRF and NBR) and much less in branch inoculated seedlings (Figure 5).

Interestingly, branch inoculated ARO eventually developed 60% wilt incidence whereas the stem inoculated ARO developed no visible symptoms. From this set of experiments it was concluded that an early season inoculation time, just after complete full leaf expansion, would probably produce the most consistent expression of symptoms.

Phenology of Leaf Emergence

Observations have subsequently been conducted to determine the period of bud break of each provenance. During the past two seasons average date of bud break has been quite similar within provenances. As a result of the earlier growing season in South Carolina, average date of bud break occurred abruptly by April 16 and was rather uniform among most species and provenances (Figure 6). Average bud break in West Virginia was approximately one to two, or more, weeks later than in South Carolina and was more variable among species and even within some provenances (Figure 7). Based on these observations, inoculations at each site should not be attempted until leaves of the slowest provenances have at least partially expanded. Even though inoculations in West Virginia will be later than in South Carolina, the more temperate growing season should ensure that symptom expression will develop to the fullest extent by late summer.

Short-term Susceptibility

In 1987 a Short-term Susceptibility Test was conducted involving one-half of the 50 trees being grown for the Long-term Susceptibility Test scheduled to be done in the mid-1990s. The purposes of this test were; 1) to develop a quantification system for estimating the amount of oak wilt symptoms, and 2) to estimate the effect of isolate source on oak wilt symptom progression.

This test was done in both states. Trees of 12 provenances representing five species were inoculated with *C. fagacearum* and observed for symptom development. In West Virginia, 100% of all trees from all provenances, including *Q. alba* and *Q. rubra* controls, developed wilt symptoms (Table 2). Symptom expression was dramatic and mortality often occurred by the end of the season (Pinon et al. 1988).

In South Carolina, only 40% of the *Q. rubra* and 18% of the *Q. alba* trees developed wilt symptoms and symptom incidence for European species ranged from 63% for *Q. pedunculata* to 6% for *Q. petrae* (Table 2). Symptom expression was slight with little mortality. Of note in the South Carolina test is that

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Figure 6. Average date of bud break in 1992 of European and American oak provenances planted at Clemson, SC.

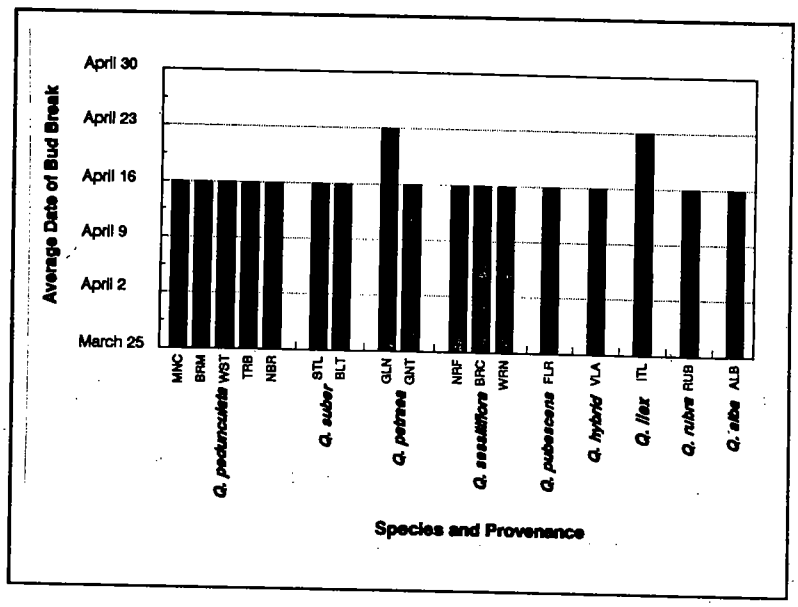

Figure 7. Average date of bud break in 1992 of European and American oak provenances planted at Morgantown, WV.

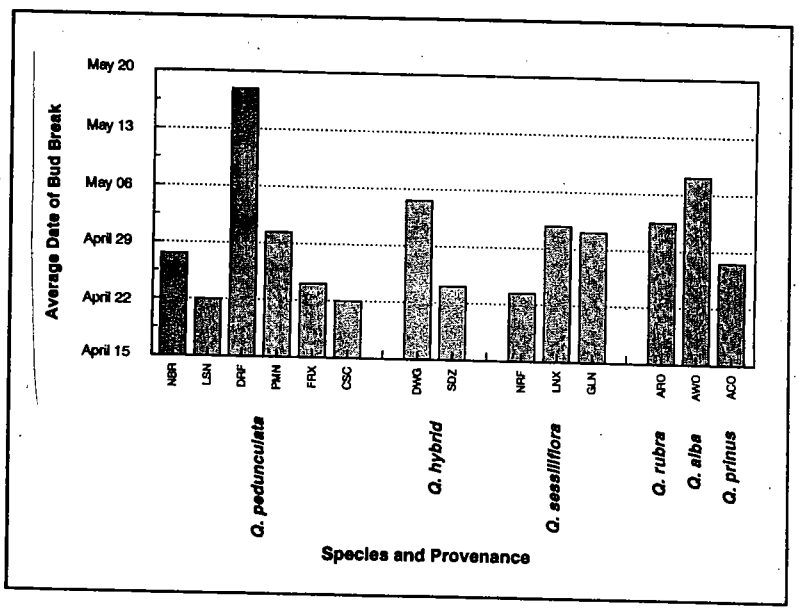

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Table 2. Average symptom development of European and American oak provenances inoculated in the Short-term Susceptibility Test.

State	1987 Symptom Rating*	1988 % Dead	1988 % Wilt Symptoms
West Virginia			
ARO	2.77	100.0	0
SDZ	2.63	97.2	0.1
LNX	2.33	89.5	1.9
NBR	2.29	83.7	2.4
FRX	2.24	91.8	2.7
LSN	1.89	78.9	0.1
DWG	1.25	80.3	5.7
AWO	0.71	11.2	4.9
South Carolina			
WST	0.40	4.5	23.8
RUB	0.33	11.1	33.4
BRM	0.33	0	45.8
TRB	0.23	0	66.7
NBR	0.19	0	74.0
STL	0.18	5.6	77.8
MNC	0.14	0	69.6

* Rating system, where;
0 = no leaves/branch symptomatic,
1 = less than 50% of branch symptomatic,
2 = more than 50% of branch symptomatic,
3 = all leaves/branch symptomatic.
All branches on each seedling were rated. An average was computed for each seedling, and then the average for each provenance was computed.

from 24 to 78% of the surviving trees developed wilt symptoms the following year.

Additional Considerations

The preliminary experiments identified some problems that still need to be addressed before conducting the Long-term Susceptibility Test. The rating system was cumbersome to use in the field and generally not very satisfactory as it inadequately described symptoms on trees with quite different crown characteristics. Some trees had very few branches and these were all relatively large. For these the system was useful. Most trees, however, had crowns comprised of few large branches, each with many small branches with many leaves. Perhaps a numerical rating system similar to that developed by Anderson and Calvert (1971) for large oak-wilted trees would be useful.

Another question was that of which inoculum source to use. All tests thus far in South Carolina had been with an isolate (SC-2) collected several

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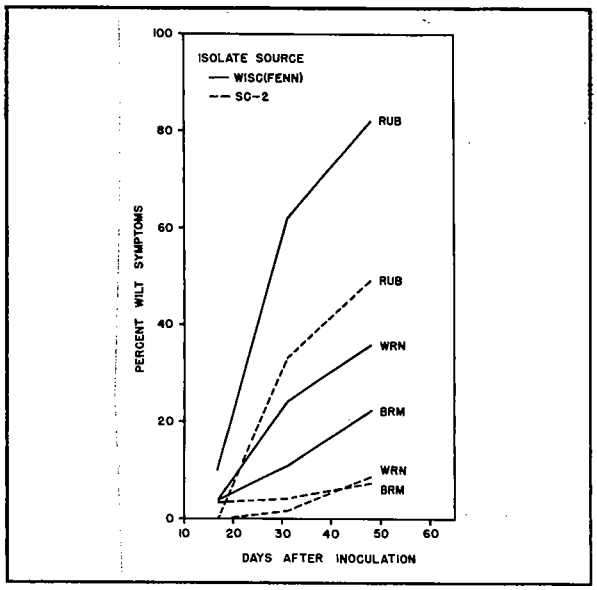
years earlier during an oak wilt survey. The West Virginia Seasonal Susceptibility Tests in 1986 incorporated a mixture of two Wisconsin isolates (Fenn and Wisc 5). The Short-term Susceptibility Test utilized a mixture of inoculum from these two plus a West Virginia (Romney 1) isolate. The differences in inoculum could account for the contrasting results obtained at both locations.

On April 30, 1988, 10-12 outplanted seedlings each of seven European and North American provenances growing in South Carolina were inoculated with one of two isolates of *C. fagacearum*. Half were inoculated with a spore suspension of a Wisconsin (Fenn) isolate, the other half with SC-2. Trees were observed during the remainder of the 1988 growing season for wilt development. The most dramatic results for three provenances are summarized in Figure 8. Note that symptom onset and speed of development were most marked for RUB, with maximum development by the Fenn isolate, being approximately double that for the SC-2 isolate at any sample time. Provenances WRN, BRM, and NRF also were more dramatically affected by the Fenn isolate. For the remaining provenances, maximum symptom development remained well below 20% for either isolate, but the Fenn isolate always produced more wilt symptoms within any provenance.

In 1989 an isolate virulence test was conducted on 13-yr-old trees of *Q. robur* growing in West Virginia. Spore suspensions were applied on June 8 to two wood chisel wounds. Results are shown in Figure 9. Wilt symptoms developed uniformly for all isolates except for SC-2 which only produced 46% symptoms by late July. After one year, however, all trees were dead except for two SC-2 inoculated trees that died later in the 1990 season.

On April 4, 1990, pole- to sawtimber-sized American red and black oaks were inoculated with spore suspensions of the same isolates used in the preceding test. In addition, a fresh South Carolina isolate (SC-7) was used as well as the older SC-2. The SC-2 isolate produced relatively minor wilt symptoms which never exceeded 10% during the growing season (Figure 10). The two Texas isolates (BAN,TX and KER,TX) produced wilt patterns at rates that also were significantly different from the patterns produced by the other isolates (Tainter and McElreath 1992).

Figure 8. Percent wilt of two European provenances (WRN and BRM) and one American provenance (RUB) following inoculation in 1988 with an isolate from Wisconsin (FENN) or an isolate from South Carolina (SC-2).



CONCLUSIONS

These results suggest that extreme care must be used in the final selection of the isolate(s) to inoculate trees in the Long-term Susceptibility Test. Not only do there appear to be effects of isolate source and age on host response, but there also may be effects of geographic location during testing. The major inoculation is planned for the mid-1990s when the trees in the arboreta are 10-13 yr old. The study of disease development should provide an accurate assessment of the susceptibility of Europe oaks to the oak wilt fungus.

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Figure 9. Percent of wilt in 13-yr-old *Q. robur* trees growing in West Virginia and inoculated on June 8, 1989 with isolates of *C. fagacearum* from sources in West Virginia, Texas, South Carolina, and Wisconsin.

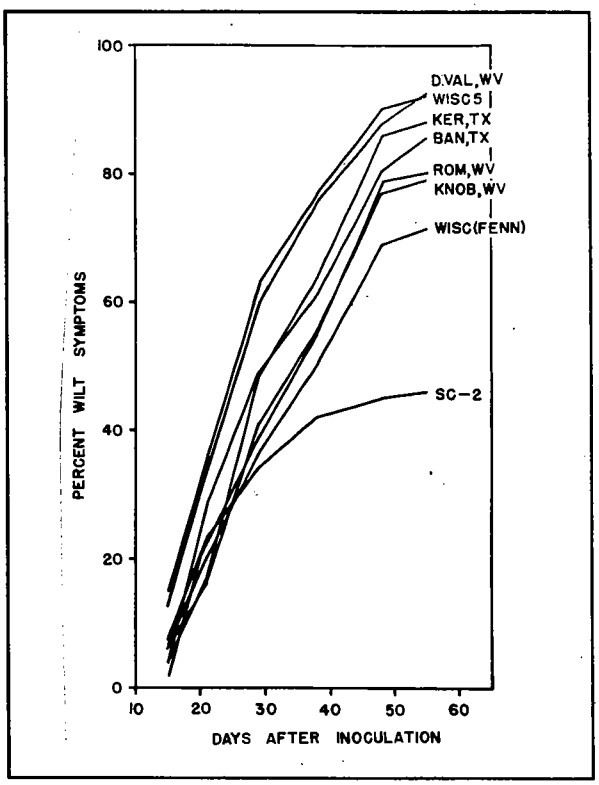
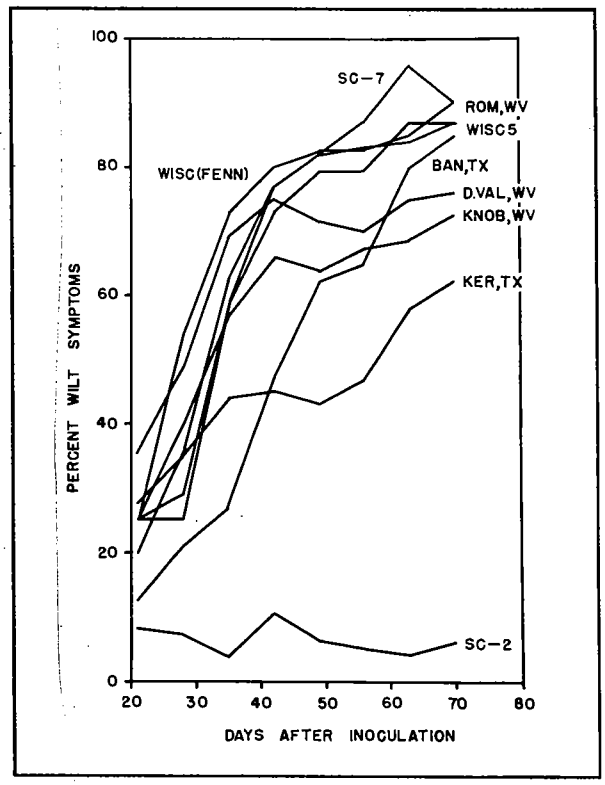


Figure 10. Percent of wilt in pole- to sawtimber-sized American red and black oaks inoculated on April 4, 1990 with isolates of *C. fagacearum* from sources in West Virginia, Texas, South Carolina, and Wisconsin. Spore counts ranged from 2.3 x 10^5 to 1.33 x 10^6, depending on the isolate.



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Section II
Current and Potential Management Practices

Live Oak Tree Improvement And Oak Wilt

Thomas A. Greene

Live oaks (*Quercus virginiana, Q. fusiformis*) are a valuable resource in central Texas. The Texas Forest Service Urban Tree Improvement Program is working to genetically improve the species by identifying and propagating superior individuals. Improvement efforts have been concentrated on increasing survival and growth in urban environments. Research in progress suggests that heritable resistance to oak wilt (caused by *Ceratocystis fagacearum*) exists in the Texas live oak population, indicating that genetic improvement of this trait is possible.

INTRODUCTION

Oak wilt (caused by *Ceratocystis fagacearum* (Bretz) Hunt) is a serious problem for live oak (*Quercus fusiformis* Small, *Quercus virginiana* Mill.) in central Texas. The pathogen is spread through root connections between adjacent trees and by insect vectors, principally beetles of the family Nitidulidae (Appel et al. 1987). Large stands of live oak in central Texas rangelands and urban areas are at risk due to their high stocking density and common root systems. Disease foci expand at a rate which is dependent on the density of live oak in the area (Appel et al. 1989).

The southeastern live oaks (series *Virentes*) have been variously described as one species or six species (Muller 1961) with varying numbers of subspecies. Vines (1960) gives two species and two varieties for members of the group in Texas. Although the taxonomy of the series *Virentes* is far from clear, it is apparent that the southeastern live oaks encompass a large amount of genetic diversity.

In addition to natural genetic diversity in the Texas live oak population, a large number of individuals from populations outside the state have been planted in urban areas within and outside of the species' natural range. This activity has created an even more diverse population which provides the genetic basis for improving live oak.

TREE IMPROVEMENT

The Texas Forest Service (TFS) has been conducting tree improvement research with urban species for two decades through the Urban Tree Improvement Program (UTIP). UTIP is a cooperative organization of commercial nurseries and municipalities who work with TFS geneticists to genetically improve native tree species for use in Texas urban environments. There are currently 18 members in the program. Since 1973, UTIP cooperators have worked with 11 species, including live oak (Table 1).

The tree improvement process as applied to live oak consisted of three phases. In the first phase, "wild" trees were chosen from among the general population on the basis of certain desirable phenotypic characteristics. These traits included apparent growth rate, limb size and angle, adaptation to severe conditions (e.g., the urban environment), and freedom from pathogens. UTIP live oak selections were made in 13 counties in Texas, as well as four parishes in Louisiana and one county in southern Mississippi. Most of these selections were made in cities since trees growing in urban areas have demonstrated the ability to survive stresses encountered in these environments.

The second phase in the tree improvement process was progeny testing. In our program, seeds were collected from the selected trees, and seedlings produced for progeny tests. The identity of the

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Table 1. Species included in the Urban Tree Improvement Program.

Common Name	Species
Live oak	(*Quercus virginiana* Mill.)
Shumard oak	(*Quercus shumardii* Buckl.)
Sweetgum	(*Liquidambar styraciflua* L.)
Bur oak	(*Quercus macracarpa* Michx.)
Southern magnolia	(*Magnolia grandiflora* L.)
Baldcypress	(*Taxodium distichum* L. Rich.)
Chinquapin oak	(*Quercus muhlenbergii* Engelm.)
Cedar elm	(*Ulmus crassifolia* Nutt.)
Bigtooth maple	(*Acer grandidentatum* Nutt.)
American sycamore	(*Platanus occidentalis* L.)
Slash pine	(*Pinus elliottii* Engelm.)

mother tree was maintained throughout the process. The male parent was unknown, since the flowers were open-pollinated. These half-sib families of seedlings were outplanted into replicated genetic tests where they were evaluated for the presence of desirable traits. For live oak, 250 selections were made and established in progeny tests.

In UTIP live oak tests, survival was assessed at age one, and height and stem diameter were measured at age three and five. These traits were used to rank all of the families in the test. Other traits have been included where they were important for a particular species; for example, UTIP selections of southern magnolia were made based on early flower production as well as growth (Greene 1991). After the families have been ranked, the top 20 percent were selected for inclusion in a seed orchard. The Urban Tree Improvement Program now has 58 superior live oak selections which were chosen on the basis of progeny test data. Scion from these superior trees has been grafted into two orchards which are being managed for seed production.

The third phase of the tree improvement process, which will begin when all of the clones in the orchard are producing seed, is orchard testing. A second set of progeny tests will be planted from seed produced in the orchard. All of these superior selections will be ranked according to data collected from these tests, and the poorer-performing bottom half of the clones will be "rogued", or removed from the orchard. This final phase will leave the best 10 percent of the original 250 selections in the orchards. The 58 live oak selections in the UTIP orchards produced seedlings which average 12.6% taller at age five than the mean of the test from which they were selected. After roguing, height improvement will be further increased.

SELECTING FOR OAK WILT RESISTANCE

If tree improvement techniques are to be used to identify sources of resistance to oak wilt, certain conditions must be met. First, genetic variability in resistance must be present in the live oak population. Second, a reliable method of detecting resistance

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must be developed. Fenn et al. (1975) developed a system for studying the response of oaks to *C. fagacearum* infection by inoculating seedlings. They listed similarities between disease development in oak seedlings and older trees and concluded that seedlings were a valid system for studying the development of oak wilt. Inoculated seedlings of the more resistant white oak (*Quercus alba* L.) developed symptoms more slowly and in smaller numbers than seedlings of northern red oak (*Quercus rubra* L.), which is quite susceptible to oak wilt as a mature tree. Further, the researchers found that development of symptoms was similar in seedlings and in older trees. However, no genetic differences in length of incubation period among northern red oak families were found.

Two experiments have been conducted to determine if genetic differences in seedling response to *C. fagacearum* infection exists within the Texas live oak population. These experiments will be described below and preliminary results will be discussed.

MATERIALS AND METHODS

Experiment 1

Acorns were collected during fall, 1988, from 20 live oak ortets equally divided among the following groups; 1) Urban Tree Improvement Program superior selections ("selections"), 2) trees whose progeny survived well but ranked low in height growth in UTIP genetic tests ("rejects"), 3) trees which were in an active oak wilt infection center, had been infected with the oak wilt fungus and suffered some crown dieback, but which had no active symptoms ("survivors"), and 4) trees just outside of the oak wilt center which had not been challenged by the pathogen ("controls"). The oak wilt center was located in Williamson County Fast growing UTIP selections and slow-growing rejects were chosen for this study because they represented the extremes in genetic variation among tested genotypes. Survivors in the oak wilt center were chosen because it was thought that their resistance might have a genetic basis. The control ortets from the Williamson County population were assumed to be genetically similar to the survivors, except that their response to oak wilt infection was unknown.

Ten seedlings from each parent tree were included in the study for a total of 200 seedlings. The ten-month-old seedlings were inoculated September 28, 1989, by a suspended drop inoculation technique (Fenn et al. 1975). Conidia of a *C. fagacearum* isolate from Bandera County were used. Seedlings were transferred to a growth chamber and kept under a regime of 13 hr dys. and 11 hr nights, with daytime temperature held at 24C° and night temperature at 21C°.

Symptom expression was noted weekly for 84 dys. and biweekly for the remainder of the study. Seedling survival was tallied 244 dys. after inoculation. To be tallied dead, a seedling had to have no living tissue in the stem or root crown. Mean incubation period and final survival percentage were calculated for each group.

The experiment was designed as 10 randomized complete blocks with each family represented by one seedling in each block. Within a block, seedlings were arranged into four row plots containing the five representatives of each group. Blocks were inoculated by one person and kept together in the growth chamber for the duration of the experiment. Data were subjected to analysis of variance, and means were separated by Duncan's multiple range test.

Experiment 2

In the second experiment, acorns were collected during fall, 1989, and grown in the greenhouse until July 2, 1990, when the seven-month-old seedlings were inoculated. Inoculation technique was identical to the first experiment, and the same isolate was used. Eight families were represented by one seedling in each of 40 replications. Replications were grouped into eight blocks (each containing five replications) for the purpose of calculating family survival. Blocks were kept together throughout the experiment. The eight families included six UTIP selections and two survivors; one selection and one survivor were common to both experiments.

Final survival was determined on October 9, 1990, 99 dys. after inoculation. Family incubation

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period and survival means within groups were subjected to analysis of variance.

RESULTS AND DISCUSSION

Experiment 1

Survival of the seedlings after 244 dys. varied with seedling group (Table 2). UTIP selections survived significantly better than Williamson County controls. More interestingly, the seedlings from Williamson County survivors also survived significantly better

Table 2. Percent survival (day 244) for four groups of seedlings inoculated with *C. fagacearum* in experiment 1.

Group	Percent Survival
UTIP Selections	88.2 a[1]
Survivors	82.0 a
UTIP Rejects	77.0 ab
Controls	62.5 b

[1] Means followed by the same letter are not significantly different at the 5 percent level, according to Duncan's New Multiple Range Test.

than the controls from the same population. It is quite likely that these differences reflect heritable characteristics peculiar to the survivors and/or the UTIP selections which could allow them to recover from oak wilt infection.

When incubation periods were compared, the survivor seedlings, which had the second highest survival rate after 244 dys., also had the shortest incubation period (26 dys.). In contrast, the selections, which had the highest survival rate, had the longest average incubation period (49 dys.). It appears that survivability of inoculated seedlings may not be directly related to length of incubation. Both traits, however, were under some genetic control.

Experiment 2

Preliminary results from this more precise experiment involving 40 seedlings in each of eight families indicated genetic diversity with respect to oak wilt symptom development existed among individuals as well as populations of live oak. Survival percentages ranged from 20 to 100% after 99 dys.. The two survivors (families 601 and 603) ranked among the best in survival but were not significantly different from the top two or three UTIP selections (Table 3). Differences existed among individual families of UTIP selections, indicating that the current tree improvement approach of individual tree selection can be successfully adapted to oak wilt resistance testing.

Table 3. Mean survival 99 dys. after inoculation for eight families of seedlings inoculated with *C. fagacearum* in experiment 2.

Family Origin	County, State	Survival
601	Williamson, TX	100 a[1]
603	Williamson, TX	95 ab
511	Grimes, TX	95 ab
547	Jim Wells, TX	90 ab
548	Brooks, TX	81 bc
523	Dallas, TX	74 c
559	Brazos, TX	58 d
537	Dallas, TX	0 e

1 Means followed by the same letter are not significantly different at the 5 percent level, according to Duncan's New Multiple Range Test.

Family heritability for survival was 0.96; that for incubation period was 0.73. Heritability is the fraction of the additive variation in a trait that is inherited when selection is performed on that trait (Baradat 1976). The large amount of variation in survival among families indicates that selection for this trait would be effective. Mean survival in

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experiment 2 was 77%, while the best three families averaged 97%.

It would be premature to relate percent improvement or heritability from seedling experiments to performance of mature trees in oak wilt centers. Before recommendations are made or large-scale screening programs are begun, more research into resistance mechanisms is needed. The role of further seedling inoculation experiments should be to identify potential sources of resistance for further study.

CONCLUSIONS

Based on this research we can conclude that; 1) genetic variability in seedling response to oak wilt inoculation exists in the Texas live oak population, and 2) the response of inoculated seedlings to oak wilt appears related to the response of mature parent trees. More research is needed to link the results of these studies to field performance of mature trees.

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Chemical Control of Oak Wilt

David N. Appel

Two compounds, thiabendazole and propiconazole, were tested for direct control of oak wilt, caused by *Ceratocystis fagacearum*. Intravascular injection with thiabendazole proved to be inconsistent and effective only on a temporary basis. In complete plot and split plot designs, crown survival in plots treated with propiconazole was greater (0 - 41% crown loss) than the levels observed in untreated control trees (61% - 100% crown loss). Preventative treatments were more effective than therapeutic treatments of trees infected at the time of injection. Propiconazole was not effective in preventing spread of the pathogen through connected roots between treated trees and adjacent trees.

INTRODUCTION

Intravascular injection of live oaks (*Quercus fusiformis* Small and *Q. virginiana* Mill.) has had a significant impact on the current recommendations for oak wilt management in Texas. Fungicide injections of an individual tree or small groups of trees complement other available management techniques in a number of different manners. As in the control of any plant disease, prevention is the best principle for ultimately controlling a pathogen.

Oak wilt prevention includes eliminating inoculum formation (fungal mats) in infected red oaks, preventing wounds on healthy oaks during the spring, applying proper wound treatments, and cautious handling of firewood. These are all aimed at preventing introduction of *Ceratocystis fagacearum* (Bretz) Hunt, the oak wilt pathogen, into an unaffected stand. Once introduced, further spread among adjacent trees may be prevented by breaking connected roots. However, trenching or other forms of barrier formation are not always feasible. This is especially true in urbanized settings. Once the alternatives have proven ineffective, injection offers the only viable tool to reduce losses of valuable trees.

The value of injection in the effort to control oak wilt goes far beyond the benefit of saving trees. Some landowners are reluctant to implement barriers when costs are high, site disruption is likely, and there is no guarantee the fungus will be stopped. Given the potential contribution of live oaks to the landscape (Martin et al. 1986), a comprehensive oak wilt management program becomes economically attractive if there are supplementary measures available to increase the likelihood of reducing further losses. There has therefore been an increased interest in oak wilt control that otherwise would not have occurred without the existence of a successful injection technique. Additionally, injection has given commercial arborists, urban foresters, and extension personnel an attractive focal point for convincing clients and cooperators to undertake ambitious, integrated control projects.

Although seemingly a recent development, injection with propiconazole (trade name Alamo, Ciba Geigy) is the result of 15 yr of testing fungicides in Texas for direct control of oak wilt. In other states, numerous antibiotics and chemicals were tested on deciduous oaks during the 1960s with no positive developments (Phelps et al. 1966). During this period, injection systems using the benzimidazoles and their derivatives were successfully used to control Dutch elm disease, caused by *Ophiostoma ulmi* (Buisman) Nannf. (Jones et al. 1973). Use of these

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compounds in deciduous oaks, however, resulted in only slight success. The acceptance of injection as a viable control for Dutch elm disease, the epidemic losses of valuable trees, and lack of suitable alternatives stimulated efforts in Texas to continue research on a reliable injection for oak wilt.

RESEARCH WITH THIABENDAZOLE

The initial experiments with benzimadazoles for oak wilt control in live oak managed to delay symptom development, but mortality rates 15 mo following injection were the same in treated and untreated trees (Lewis and Brook 1985). Speculation on the cause of the failures focused on poor distribution of the fungicide in the tree rather than a failure on the part of the compound to inhibit growth of the pathogen. Further testing and demonstrations achieved similar delays in symptom development (J. Johnson, personal communication). Public concern for oak wilt was sufficient to gain emergency registration of thiabendazole (Arbotect®, Merck) injection for disease control, in spite of tenuous results.

In order to improve tree survival in injected trees and clarify reasons for failures, the Forest Pathology Lab at Texas A&M University initiated systematic injection experiments with Arbotect® in the early 1980s. These studies focused on uptake and distribution of thiabendazole (TBZ) and survival of treated trees under conditions of natural infection. A brief discussion of these experiments is presented below.

Materials and Methods

Arbotect® 20S was used from 1982 - 1987 for injection of live oaks growing in rangelands and under urban conditions. A modified garden sprayer was used to inject high volumes of fungicide solutions (11 fungicide solution/in. dbh = diameter-breast-height) into the exposed root flares. The injection apparatus consisted of the 11l, hand-pumped garden sprayer connected to plastic tubing and injection ports. Each port was inserted into a 7 mm (5/16 in.) hole drilled through the bark and approximately 2.5 cm (1 in.) into the sapwood. Holes were drilled on 10 - 15 cm (4 - 6 in.) spacings, and the solutions were injected at 20 - 30 p.s.i.

Treatment plots were chosen near actively expanding disease centers. Preventative treatments were applied to symptomless trees growing adjacent to, or within 33 m (108 ft) of, symptomatic trees. Trees treated therapeutically were in various stages of crown loss. The types and extent of foliar symptoms on the therapeutic treatments ranged from vein banding and veinal necrosis to non-descript patterns of chlorosis and necrosis. Plots located on the Robinson ranch near Round Rock, TX, were randomly selected following a 10% line-plot survey through a large disease center (Appel 1986). Groups of trees growing in the yards of Austin neighborhoods served for additional treatments.

Periodically, the trees were observed to compare rates of disease progress in treated trees with the untreated controls. Disease progress was measured in three manners, including; 1) the extent and types of foliar symptoms, 2) the degree of crown loss, and 3) numbers of dead trees.

Thiabendazole levels were estimated in twigs and branches removed from injected trees using a bioassay system. Spore suspensions of *Penicillium expansum* were sprayed over the surface of agar on which debarked twig samples were placed. After incubation for two dys., fungitoxicity was determined by measuring the zone of fungal inhibition formed as a result of diffusion of the fungicide into the agar surrounding the twig sample. The presence of fungicide also could be observed by inhibition of fungal growth on the surface of the twig. Details of this method are described in Nishijima and Smalley (1979). The water relations of injected trees were estimated utilizing pre-dawn measurements taken with a Schollander pressure chamber.

Results and Discussion

The results of the TBZ injections over the five yr period indicated that a high degree of variability existed in survival of trees following treatment and oak wilt infection (Roberts 1988). There was some reduction in the rate of mortality among the therapeutically-injected trees when compared to the untreated controls, but results were not sufficiently clear to lend any confidence in recommending therapeutic injections with TBZ. Also, trees injected prior to infection became diseased at lower rates than

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in the uninjected controls, but the preventative effect was only temporary. Given these results and those from numerous other experiments, the use of Arbotect® 20S for oak wilt prevention decreased dramatically and by 1986 was no longer distributed in Texas.

In spite of the discouraging efficacy data, a great deal of valuable information was obtained over the course of the experiments with TBZ. In addition to survival, distribution of the fungicide also was found to be highly variable, ranging from 11% to 100% of the samples bioassayed from tree crowns. A seasonal influence was detected, with best distribution occurring following the June injections. The poorest distribution followed injection in December. Tree health and environmental conditions were found to have a weak influence on rates of uptake, but had no influence on the distribution of TBZ in the tree crowns.

Several important criteria were formulated to serve as guidelines in subsequent experiments to evaluate intravascular injection with fungicides for oak wilt control in live oak. Rapid rates of mortality caused when the fungus colonizes common root systems provides a unique opportunity to measure disease progress under highly predictable, natural conditions for infection. Due to a poor understanding of latent infections in symptomless trees, trees used in preventative treatments presumed to be healthy were in reality already diseased, so that experiments needed to be designed with sufficient numbers of trees located at varying distances from the perimeter of the disease center.

Another complicating factor was the variability in mortality rates observed among plot locations. Complete mortality in live oak was common, but at least 10 - 20% survival of untreated trees with varying degrees of crown loss could be expected. Control plots were particularly important for demonstrating the effectiveness of a treatment in raising the survival rates beyond those observed in representative, untreated trees. These conclusions were valuable in designing further experiments for testing additional, promising fungicides.

RESEARCH WITH PROPICONAZOLE

Propiconazole (PPZ) represented a new class of compounds, the triazoles, which became widely accepted for a variety of crop diseases during the 1980s. The triazole fungicides have many superior traits for tree injection when compared to TBZ. Acting as ergosterol-biosynthesis-inhibitors (EBIs), the triazoles are highly systemic, low in phytotoxicity, and fungistatic at extremely low dosages. The high levels of destruction caused by the oak wilt epidemic and disappointing results with TBZ warranted testing of propiconazole for controlling the losses of live oaks.

Materials and Methods

The injection apparatus and operating conditions were similar to those used in research with TBZ. Initial experiments with TBZ clearly demonstrated that trees located on the perimeters of actively expanding infection foci were the best candidates for treatment. Two types of plots were used during testing with PPZ to compare the response of treated trees to uninjected trees naturally infected by the pathogen. These were; 1) complete plots, 2.0 ac or less in size, consisting of trees which were all treated similarly within the plot, and 2) split plots, where treated trees were intermingled in the same plot with untreated trees. Symptomatic as well as asymptomatic trees located immediately adjacent to trees with symptoms were considered to be candidates for therapeutic injections.

An attempt to quantify the level of risk was made by measuring the distance between the treated tree and the nearest symptomatic tree and combining a "risk factor" to the tree being injected. This risk factor was based on the spatial arrangements of the trees, with particular regard to the distance to the nearest symptomatic live oak. A diseased tree in a plot was rated 0, a symptomless tree immediately adjacent to a diseased tree was rated 1, and the next symptomless trees in line were rated 2, 3, 4, etc. Average values for the distances measured and risk factors assigned are given in Table 1.

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Table 1. Locations and tree attributes for plots and sub-plots containing native live oaks used for testing the efficacy of PPZ for control of oak wilt in central Texas.

Plot No.	No. Trees	DBH[x] (in.)	Distance[y]	Risk Factor
		Injected		
1	8	11.3	43.0	2.9
2	8	8.3	21.5	1.4
3	6	17.3	53.0	1.5
4	9	16.4	51.8	1.1
5	14	19.9	27.6	1.5
6	6	25.4	45.0	1.0
7	6	12.9	57.5	1.5
		Uninjected		
1	8	6.1	43.6	3.6
2	8	11.4	21.6	1.0
7	6	14.0	49.2	1.7
8	6	-[z]	31.2	3.5
9	15	7.0	-	-

[x] DBH = diameter breast height, as the average for all trees in the plot
[y] This figure is the average distance of all the trees in the plot from a symptomatic tree.
[z] - Indicates no measurements taken

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Table 2. Tree characteristics and disease progress for four symptomatic and four asymptomatic trees injected with propiconazole in plot 2.

Tree Number	DBH (in.)	Risk Factor	Distance (ft.)	% Crown Loss				
				0 mo.	8 mo.	13 mo.	20 mo.	27 mo.
				Asymptomatic				
1	9.7	4	63	0	0	0	5	30
2	6.6	3	37	0	0	0	0	0
3	6.5	2	37	0	0	0	40	10
4	10.9	2	35	0	10	10	50	35
				Symptomatic				
5	10.9	0	-	0	0	20	40	45
6	7.0	0	-	0	0	30	60	35
7	7.4	0	-	0	0	30	40	35
8	7.4	0	-	0	0	40	40	30

Propiconazole was injected at rates of 1 - 3 ml/in. dbh/l H_2O. The concentration of this mixture ranged from 115-45 ppm, whereas the solubility limit for PPZ is 115 ppm. At these rates, a white precipitate collected in the injection tubing during the first few hours of treatment. Depending on the size and health of the tree, the process was usually complete within 4-5 hr. Large, diseased trees sometimes took up to 24 hr to complete. After one day, the process was discontinued and the tree was presumed to be in the advanced stages of colonization.

Results and Discussion

Following the TBZ experiments, substantial consideration was given to establishing useful experimental plots for testing with PPZ. Treated trees and uninjected trees were at the same risk of infection by *C. fagacearum* (Table 1). There were no meaningful differences between treated and untreated plots in the average distances from the trees in the plot to the nearest infected tree. Plots 2, 5, and 6 contained a few symptomatic trees, giving the opportunity for therapeutic as well as preventative treatments. Average diameters also were similar for trees in the experimental plots.

Significant differences were observed in the disease ratings within one year following treatment and natural challenge of the trees by the oak wilt pathogen. In Figure 1, the differences in percent crown loss can be seen for treated and control trees in the split plots (no. 1, 2, and 7). In every plot, average crown loss was greater in the uninjected trees. Similar observations were made in the complete plots (Figure 2). The two uninjected plots (nos. 8 and 9) had significantly greater crown loss than the four treated plots (nos. 3, 4, 5, and 6). The same trend was observed for tree mortality. Within three years following treatment a total of five out of 57 trees (9%) had died in the injected plots, while 30 of the 43 control trees had died (70%). Based on these results, PPZ was judged to be an efficacious treatment for oak wilt control in live oak and was subsequently registered for use in Texas.

The injection experiments were designed to clearly establish the utility of propiconazole for preventative control of oak wilt. However, detailed analysis of the results provides further insight on the

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Figure 1. Levels of disease (percent crown loss) in three split plots consisting of trees injected with propiconazol and untreated trees infected with *C. fagacearum*.

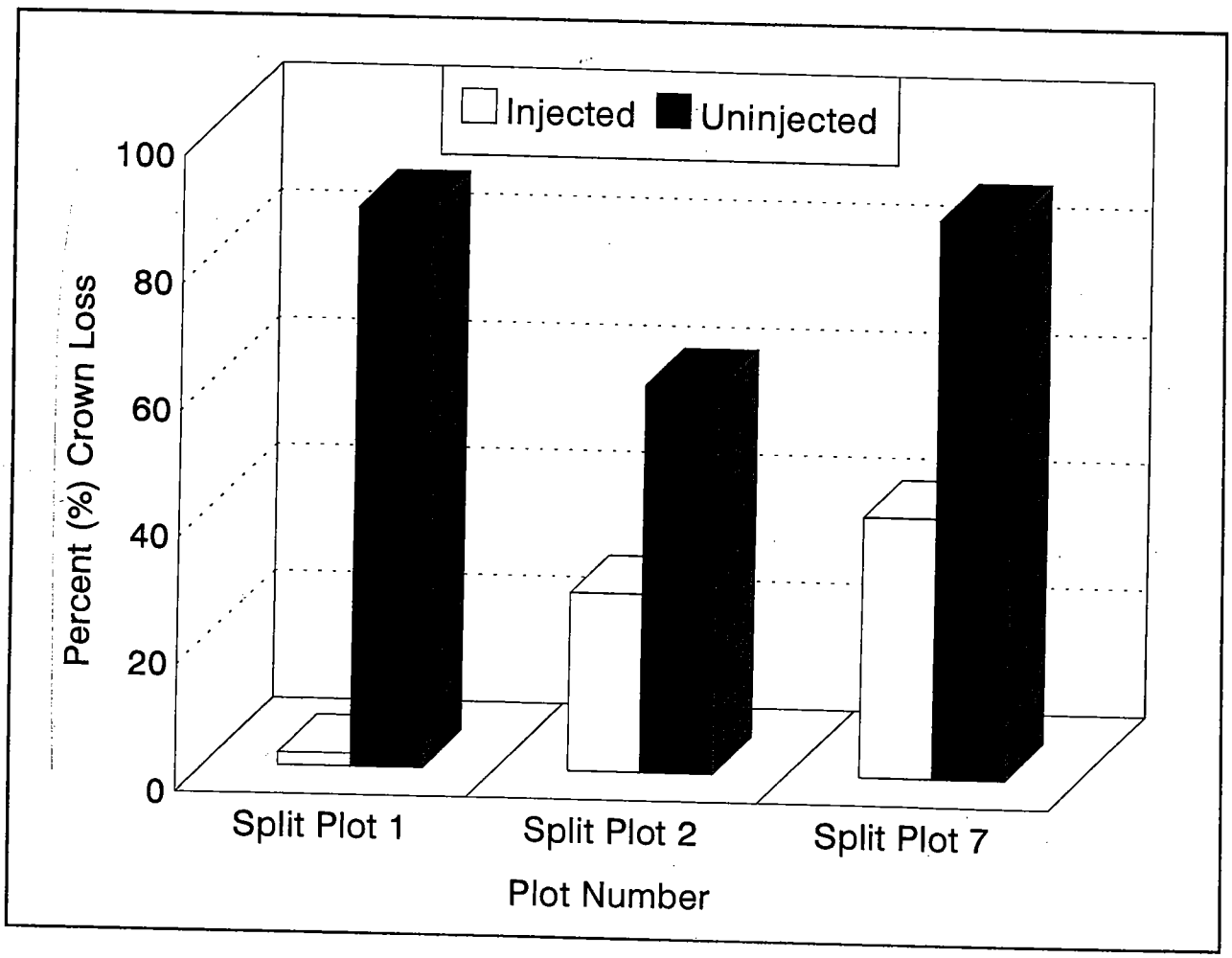

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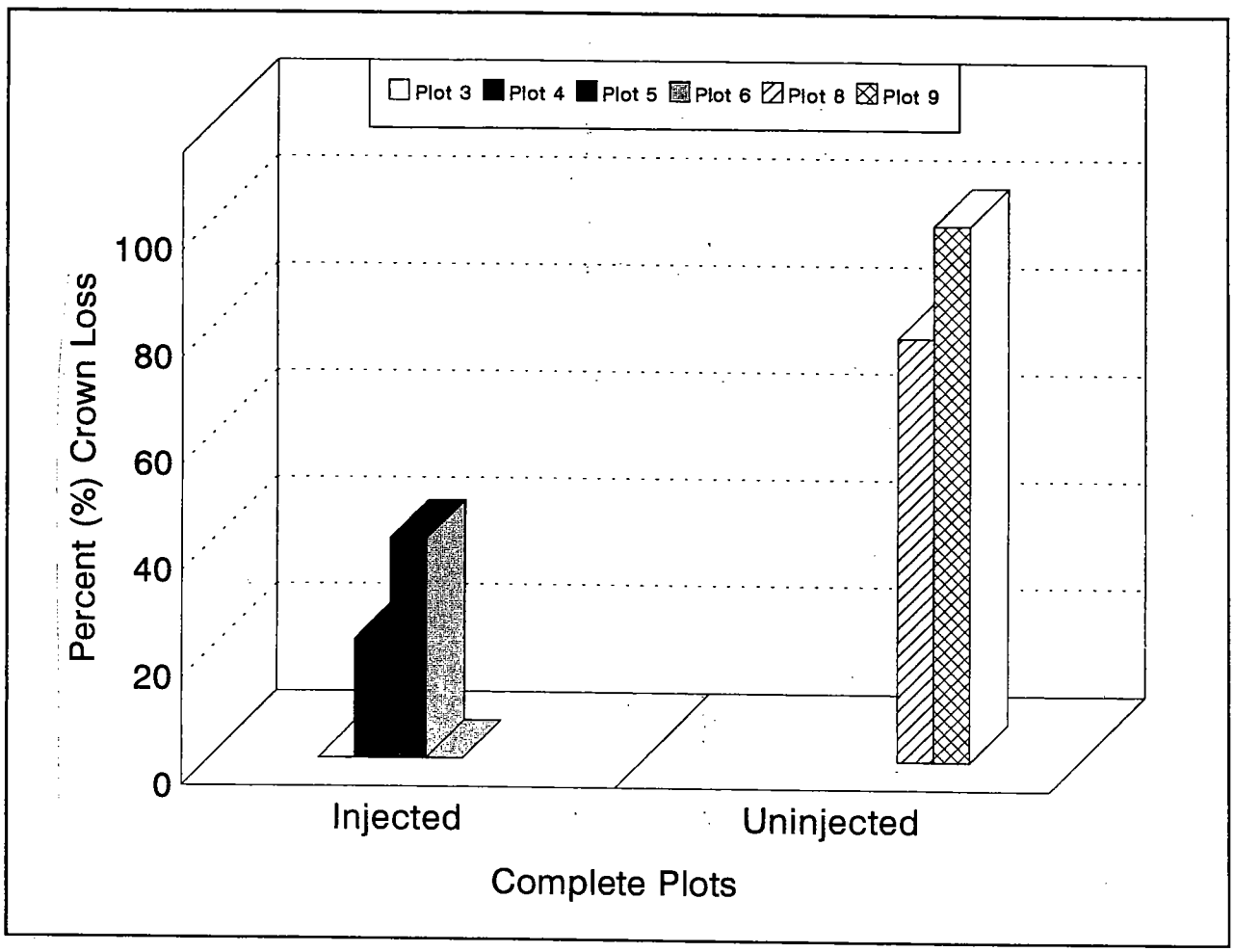
Figure 2. Levels of disease (percent crown loss) in 6 complete plots consisting of trees injected (plots 3, 4, 5, and 6) with propiconazole and uninjected trees (plots 8 and 9).



treatments. For example, in split - plot 2, four of the injected trees were injected preventively while the other four were symptomatic at the time of treatment, thus receiving therapeutic treatments (Table 2). Although these trees had scattered oak wilt symptoms, they were not yet sustaining any discernable degree of crown loss. Disease progress in the asymptomatic trees was greatly reduced over that observed in the symptomatic trees in plot 2 (19% vs. 36%, respectively). These results demonstrate that PPZ appears to inhibit colonization of trees already infected by the fungus, although the degree of protection is not nearly as great as in the preventative treatments. In some cases, preventative treatments were completely effective in preventing crown loss, but trees usually became symptomatic and sustained some low degree of damage. This observation was particularly critical in formulating a strategy to deploy injection in conjunction with other control methods. Injected trees at high risk to infection are

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likely to become diseased, and will pass the fungus on to adjoining trees in spite of treatment. Therefore, injections should always be used in an integrated approach rather than alone to reduce losses in live oak from oak wilt.

CONCLUSIONS

Tree injection for control of vascular pathogens has been a controversial and sometimes questionable practice in the field of forest and shade tree pathology. The wounding required for the injection process disrupts a tree's vascular system and results in eventual decay and discoloration. Many of the compounds originally tested for intravascular injection were highly phytotoxic and achieved inconsistent results. The results of testing with PPZ for oak wilt control in live oak indicate that injection may be a viable tool, depending on the compound being used. In addition to the attributes mentioned previously, PPZ has growth regulator properties that may elicit valuable host responses toward the pathogen (Kuck and Scheinpflug 1986). Although injection may be harmful to a tree, the high mortality rate caused by *C. fagacearum* and the positive effects of treatment with PPZ have made intravascular injection an important tool in the control of oak wilt in live oak.

Another advantage that may contribute to the success of PPZ to control oak wilt is the apparent ability within live oaks to partially resist infection by *C. fagacearum* and survive, to some degree, with no treatment. Such variable, low-level survival rates are not exhibited by deciduous red oaks. Red oaks may be too susceptible, when compared to live oaks, for injection with PPZ to have any positive influence on the disease process. Further testing with PPZ on deciduous red oaks is currently underway at the University of Minnesota.

There are certainly limitations to the degree of control that may be achieved with intravascular injection. These limitations may be overcome, in part, by studying the uptake and distribution of PPZ in injected trees. Studies with TBZ clearly demonstrated there is a seasonal influence on the distribution of compounds in crowns of trees injected at different times of the year. Similar research is needed with PPZ. PPZ also is extremely inhibitory towards the Dutch elm disease pathogen, *Ophiostoma ulmi* (Buisman) Nannf (Appel and Kurdyla 1992). This and other related triazoles may hold new opportunities for the control of many, previously intractable, tree diseases.

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Biological Control of Oak Wilt

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D. N. Appel
D. S. Brooks
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Endophytic bacteria were isolated from live oak trees (*Quercus fusiformis*) representing three Texas oak wilt centers and evaluated for *in vitro* inhibition of *Ceratocystis fagacearum*. Six representative isolates were evaluated for their ability to colonize both Spanish oaks *(Q. texana)* and live oak trees. *Bacillus* species and *Pseudomonas* species were better able to colonize Spanish oaks and live oaks, respectively. Pretreatment of live oaks with *P. denitrificans* strain 1-15 significantly reduced the number of diseased trees in one experiment and significantly reduced crown loss in another. Pretreatment of live oaks with *P. putida* strain 5-48 did not significantly reduce the number of diseased trees but did significantly reduce crown loss in one study. Evaluation of selected bacteria to colonize and survive in mature, naturally-grown live oaks showed that introduced bacterial isolates were distributed in the vascular tissue, but did not readily colonize the xylem. Additionally, it was determined that isolate 1-15 can persist up to one year after introduction in live oak, but was not found distributed throughout the tree. Preliminary results indicate that the treated trees were not protected; final results are pending.

INTRODUCTION

Oak wilt is a vascular disease of oak trees incited by the fungus *Ceratocystis fagacearum* (Bretz) Hunt. In central Texas, an oak wilt epidemic is devastating live oak trees (*Quercus fusiformis* Small and *Q. virginiana* Mill.) in urban as well as rural areas (Appel and Maggio 1984). Current suppression efforts have focused on controlling transmission of the pathogen by physical isolation of trees in disease centers from healthy trees and chemical treatment of infected and at-risk trees.

Methods to prevent root transmission of the pathogen include trenching around diseased oaks and removal of host trees within 100 ft of diseased oaks. Removal of diseased red oaks (*Q. texana* Small) prevents formation of fungal mats. These are the source of inoculum for the long distance transmission of the pathogen by insect vectors, including nitidulids or sap beetles (Coleoptera: Nitidulidae).

Recent research conducted using chemical fungicide injections of propiconazole into the vascular systems of oak trees to control or prevent oak wilt have improved the potential outlook for control (Appel 1990). However, the potential for cancellation of specific fungicides by the Environmental Protection Agency and their persistence in the environment warrants the investigation of alternative strategies for control. An appropriate alternative strategy for control could include biological agents.

Biological control has been traditionally defined as the use of living organisms as pest control agents (Scheffer et al. 1989). The National Academy of Sciences Research Briefing Panel has defined biological control as "the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favor desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms" (Garcia et al.

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1988). It is in this context that the term biocontrol will be used for the purpose of this presentation.

Biological control has been studied in other vascular wilt diseases of trees such as Dutch elm disease (DED, caused by *Ophiostoma ulmi* (Buisman) Nannf.) (Myers and Strobel 1983, O'Brien et al. 1984, Scheffer 1983, Shi and Brasier 1986) and verticillium wilt of maples (caused by *Verticillium dahliae*) (Hall et al. 1986). Microorganisms that have shown *in vitro* inhibition of both the DED and verticillium wilt pathogens include *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp., and *Streptomyces* spp.(Gregory et al. 1984, Hall et al. 1986, Meyers and Strobel 1981, Murdoch et al. 1984, Strobel and Meyers 1981).

Several reports indicate *in vitro* inhibition of *O. ulmi* by several species of *Pseudomonas* (Gregory et al. 1986, Meyers and Stroble 1981, Murdoch et al. 1984, Scheffer 1983, Shi and Brasier 1986), and biological control of DED has focused on the use of these microorganisms as potential agents for control (Meyers and Strobel 1981, Scheffer 1983, Shi and Brasier 1986, Strobel and Meyers 1981). Attempts to control DED using introduced bacteria as biological control agents in trees have met with varying results (Meyers and Strobel 1983, Scheffer 1983, Shi and Brasier 1986).

We report here on the isolation, characterization, and evaluation of endophytic bacteria as potential biocontrol agents for the control of oak wilt. Portions of this research have been previously presented (Brooks et al. 1988a, b, Gehring et al. 1990).

MATERIALS AND METHODS

Media and Storage

The following growth media were used in the experiments as described below:

- *Bacillus* sporulation agar (Brooks 1989)
- Nutrient broth-yeast extract agar (NBY) (Vidaver 1967)
- King's medium B (Henry 1944) potato dextrose agar (PDA) (Difco)
- Tryptic soy agar (BBL)
- Tryptone nutrient (TN) medium (Olsen and Hansen 1976)

To obtain a solid medium (TNA), TN was amended by adding 20 g of agar per liter. For broth (TNB), 2 g of KNO_3 was added per liter to the basic TN medium. Short- and long-term storage of bacterial isolates were as described by Brooks (1989).

Bacterial Isolation and Identification

Three active oak wilt centers located in central Texas were used as sources of bacterial isolates. The first site was located eight miles south of Round Rock (Williamson County), the second site was located near La Grange (Fayette County), and the third site was located at Kerrville State Park in Kerrville (Kerr County). Four live oak trees located in the interior of an infection center at each site were selected for sampling. Two of the trees sampled were considered as disease "escapes," whereas the others showed symptoms of previous oak wilt infection, but were in apparent remission from the disease.

Each oak wilt center was sampled four times during a one-yr period by removing two branch samples and two bole samples. The samples were transported to the laboratory on ice and processed as described by Brooks (1989). Bacterial colonies from the sapwood were given strain designation numbers according to the date, site, and source from which they were isolated. Isolates were purified using a streak dilution method and single colonies transferred to NBY plates for storage and further analysis. Bacterial isolates were identified by fatty acid methyl ester profile analysis using the Hewlett-Packard Microbial Identification System (MIS) (Miller and Berger 1985, Moss et al. 1974).

In Vitro Inhibition Assay

Evaluation of *in vitro* inhibition of *C. fagacearum* by the bacterial isolates was accomplished as described by Brooks (1989). Media containing low to high concentrations of available iron were employed to aid in classifying the type of inhibitory compound(s) produced. Inhibition on the low iron medium (KB) indicated siderophore-like compound(s) whereas inhibition on the high iron, low phosphate medium, PDA, indicated antibiotic-like compound(s). The sizes of inhibitory zones were measured from the edge of

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the bacterial colonies, and the type of media on which the bacteria produced the zones was recorded.

Tree Injection

All preparation of inoculum and injections for the colonization and challenge studies were performed as described by Brooks (1989). A modified Sterrett-Craeger microinjector system was used to deliver the inoculum (Sterrett and Creager 1977).

Selection For Antibiotic Resistant Bacterial Isolates

Antibiotic-resistant segregants of the selected bacterial isolates were obtained by plating the isolates to a series of TNA plates amended with increasing amounts of antibiotic (streptomycin or nalidixic acid). Bacterial strains were selected for growth at an antibiotic concentration of at least 200 µg/ml of medium. The resistant isolates were streaked for isolated colonies, retested for *in vitro* inhibitory properties, and stored as previously described (Brooks 1989).

Colonization Studies

To allow for the isolation of selected bacteria from sapwood, antibiotic resistant segregants of the bacterial isolates were used. Bacterial isolates used in this study are listed in Table 1. To evaluate colonization, the bacterial isolates were injected at concentrations of 10^5 and 10^8 cfu/ml. One ml of each of the bacterial concentrations was injected into nine live oak and nine red oak trees with control trees injected with phosphate buffer. The qualitative assay for colonization was as described by Brooks (1989).

Challenge Studies

Based upon the *in vitro* inhibition and colonization studies, several bacterial isolates were selected for *in vivo* fungal challenge studies. The challenge studies consisted of injecting a bacterial suspension into containerized oak trees and challenging the trees with injections of *C. fagacearum* one week later. The bacterial suspension was prepared, as previously described, at a concentration of 10^8 cfu/ ml (Brooks 1989). Each bacterial isolate was injected (1 ml) into 10 trees. Control injections consisted of sterile phosphate buffer. Each tree was injected with one ml of a conidial suspension at 10^6 spores/ml seven dys. after the bacterial injections. The trees were randomly distributed in the greenhouse to allow for statistical analysis. The trees were numbered and monitored for disease symptoms. As disease symptoms developed, disease progression was recorded for each tree as percentage crown loss. Disease progression was recorded for eight weeks. At the termination of the experiment, six trees were assayed to determine persistence of the bacterial isolates in the sapwood tissue. All challenge experiments were conducted under greenhouse conditions.

Colonization Studies in Mature Live Oak Trees

The bacterial isolates used in the injections have been described in previous reports (Brooks 1989). The bacterial isolates used were identified as *Pseudomonas denitrificans* (1-15), *Bacillus pumillus* (1-1), and *Pseudomonas putida* (Miss Y-20). Five 0.10 ac (0.04 ha) plots were established on the Robinson Ranch in Round Rock, Texas for the colonization study as described by Gehring (1990). The ranch is located on highway 620, 107 mi (171 km) from Texas A&M University and consists of large, homogenous stands of live oak. A grid was previously established on a 4 x 5 chain survey (1 chain = 66 ft (19.8 m)). Plots were located at positions 8A.5, 8A-1, 7.5A, 8A, and 6A on the survey lines. The plots were located on the perimeter of an actively progressing disease center. Individual trees were chosen based on their distance from dead or diseased trees. This placement of the treated trees was designed to allow natural challenge of the treated trees within 1-3 yr following treatment. Inoculum, injection, and sampling protocols were as described by Gehring (1990).

Characterization of Fungitoxic Compound

The extraction procedure was as described by Gehring (1990). An arbitrary unit of fungal inhibitory compound (FIC) was defined as that amount needed in an end-point dilution to completely

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inhibit the growth of *C. fagacearum* growing on PDA when applied in a 20 µl sample.

Effects of FIC on Spore Germination

Studies were conducted to determine the effects of the fungitoxic compound on the germination of *C. fagacearum* conidia. Forty of FIC were added to a conidial suspension with final concentrations of 0, 1, 2, 4, 8, and 16 units/ml. Conidial suspensions consisted of one ml of 10^6 washed conidia and one ml of PDB. The mixtures were incubated at room temperature for 18 hr and the percentage of germinated spores determined from four individual 100 spore counts of each of the respective treatments.

RESULTS AND DISCUSSION

Isolation and Evaluation of Bacterial Isolates

A total of 889 bacterial isolates were obtained during the sampling periods from the sapwood tissue of live oak trees in three oak wilt centers located in central Texas. Of this total, 183 or 20.5 % showed *in vitro* inhibition of *C. fagacearum*. The bacterial strains produced either a siderophore-like compound(s), antibiotic-like compound(s), or both, with zones ranging from 1 mm to 20 mm. A total of 70 bacterial isolates obtained from Round Rock samples (32 from escape trees and 38 from remission trees) showed fungal inhibitory activity (FIA) against *C. fagacearum*. The isolates included three genera; *Bacillus*, *Erwinia*, and *Pseudomonas*.

Forty-two bacterial isolates exhibiting FIA were isolated from the La Grange site (20 from escape trees and 22 from remission trees). Inhibitory bacteria isolated from the La Grange site included three genera; *Bacillus*, *Erwinia*, and *Pseudomonas*. Samples obtained from the Kerrville site yielded 71 bacterial isolates (42 from escape trees and 29 from remission trees) that exhibited *in vitro* inhibition of *C. fagacearum*. The inhibitory bacteria isolated from Kerrville were identified as members of four genera; *Bacillus*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Bacillus* spp. and *Pseudomonas* spp. were the most common species identified from both remission and escape tree samples obtained from all three sites.

Colonization Studies

Based on the preliminary *in vitro* inhibition testing and identification of the bacterial isolates from live oak trees, six strains were selected for evaluation as potential biological control agents. The strains and selected characteristics are listed in Table 1. Since a necessary feature of a biocontrol agent would be its ability to colonize the functional tissue of a tree, the six isolates were evaluated for their ability to be distributed in sapwood tissue of container-grown red and live oak trees. Control trees were evaluated for resident populations of bacteria that might interfere with the distribution of the introduced bacterial isolates. None of the resident microbial population showed *in vitro* inhibition of the selected bacterial isolates or *C. fagacearum*. Injections were accomplished using antibiotic resistant segregants of the selected strains and their distribution was monitored.

In container-grown red oaks inoculated with bacteria at the 10^5 cfu/ml concentration, all the introduced isolates appeared to distribute equally on initial introduction. However, none of the isolates appeared to persist or distribute into the root system (Figure 1A).

The average mean distribution of the introduced bacterial isolates in the live oak trees injected at the 10^5 cfu/ml above the injection point was 15.67 cm $\pm$ 4.20 cm at day 0 (Figure 1B). The *Bacillus* spp. (Figure 1B, bars A,C, and E) showed the best initial distribution, whereas the Gram-negative isolates showed lower primary distribution (Figure 1B, bars B, D, and F). However, isolates 1-15 and 5-48 showed good persistence and distribution into the root system at week four which may be indicative of their colonizing ability in this system (Figure 1B). The Gram-positive isolates had a lesser distribution after four wk in live oaks.

Figure 1C shows the distribution using an inoculum concentration of 10^8 cfu/ml on day 0 and week four in the red oak trees. The initial distribution of the bacterial isolates at 10^8 cfu/ml was not significantly higher than that observed with 10^5 cfu/ml injections. However, the two *B. pumilis* isolates were found to distribute in the red oaks to a significantly higher degree than the other isolates as

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Table 1. Bacterial isolates evaluated for distribution in oak trees.

Bacterial Isolate	Designation (Gram rxn)	Type of Inhibitory Compound[1]	Source[2]
B. pumilus	1-1 (G+)	S (4), A (11)	R, M
P. denitrificans	1-15 (G-)	S (10), A (17)	R, E
B. pumulis	3-23 (G+)	S (0), A (7)	R, M
P. putida	5-48 (G-)	S (30), A (0)	L, E
B. alvei	6-27 (G+)	S (6), A (0)	L, E
E. herbicola	9-16 (G-)	S (0), A (20)	K, M

[1] S = siderophore-like compound as determined on King's medium B,
A = antibiotic-like compound as determined on PDA; number in parenthesis indicates the size of inhibitory zone in mm from edge of the bacterial colony.

[2] Source of bacterial isolate from initial isolation studies, where R = Round Rock, L = LaGrange, K = Kerrville, E = escape tree, M = remission tree.

indicated by samples assayed at week four (Figure 1C, bars A and C).

In the live oaks injected at the 10^8 cfu/ml concentration, the average mean distribution of the introduced bacterial isolates above the injection point was 19.6 cm $\pm$ 3.2 cm at day 0. Initial distribution of the bacterial isolates was not significantly higher at 10^8 as compared to the 10^5 injections. However, after four wk the persistence of the Gram-negative isolates was higher overall than that observed for the Gram-positive isolates. Additionally, the Gram-negative isolates were found to more readily colonize the root systems.

In general we have observed, in this and other studies, that the *Bacillus* spp. are more readily distributed and persist in red oaks. Alternatively, *Pseudomonas* spp. distribute better in live oaks and appear to colonize the root system.

Challenge Experiments

Studies designed to evaluate the potential distribution of bacterial isolates allowed for the further selection of isolates to be evaluated as possible control agents. Several isolates were evaluated for *in vivo* inhibition of the oak wilt pathogen. Experiments were designed to evaluate the prophylactic efficacy of colonizing bacteria for the reduction of disease incidence and symptom expression. Two separate experiments were conducted using container-grown live oak trees, to test the potential protecting capacity of selected bacterial isolates to a direct challenge by the oak wilt pathogen. The isolates selected for the live oak experiments were *P. denitrificans*, isolate 1-15 and *P. putida*, isolate 5-48. Live oak trees treated prophylactically with isolate 1-15 showed a significant ($P \leq 0.05$) reduction in disease incidence and displayed an average crown loss of 25% in the first experiment (Table 2).

No reduction in the incidence of disease was observed in the second experiment, but a significant reduction in crown loss was observed in trees treated with isolate 1-15. Trees treated with isolate 5-48 showed less disease than controls in the first treatment and significantly less crown loss in the second experiment (Table 2).

One experiment was conducted to evaluate the prophylactic treatment of container-grown red oak trees to challenge by the oak wilt pathogen. The two isolates chosen for this experiment were *P. denitrificans* 1-15 and *B. pumilus* 1-1. All 10 trees in

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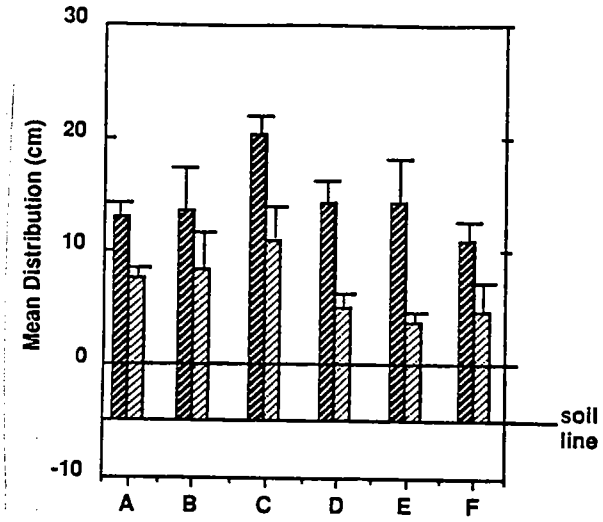

Figure 1A. Distribution of bacterial isolates from point of inoculation in sapwood tissue of container-grown red oak trees. Average tree height from soil line was 75.28 cm. A) *B. pumulis* 1-1; B) *P. denitrificans* 1-15; C) *B. pumulis* 3-23; D) *P. putida* 5-48; E) *B. alvei* 6-27; and F) *E. herbicola* 9-16. ▨ - Day 0; ▨ - Week 4. Inoculum was 10^5 cfu/ml. Bars represent standard error of the mean.

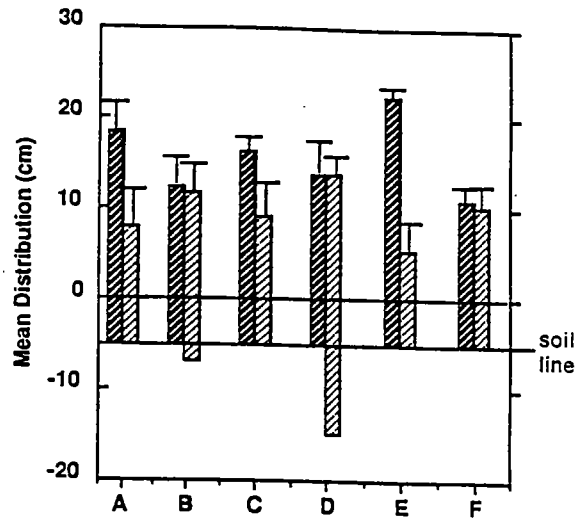

Figure 1B. Distribution of bacterial isolates from point of inoculation in sapwood tissue of container-grown live oak trees. Average tree height from soil line was 73.17 cm. A) *B. pumulis* 1-1; B) *P. denitrificans* 1-15; C) *B. pumulis* 3-23; D) *P. putida* 5-48; E) *B. alvei* 6-27; and F) *E. herbicola* 9-16. ▨ - Day 0; ▨ - Week 4. Inoculum was 10^5 cfu/ml. Bars represent standard error of the mean.

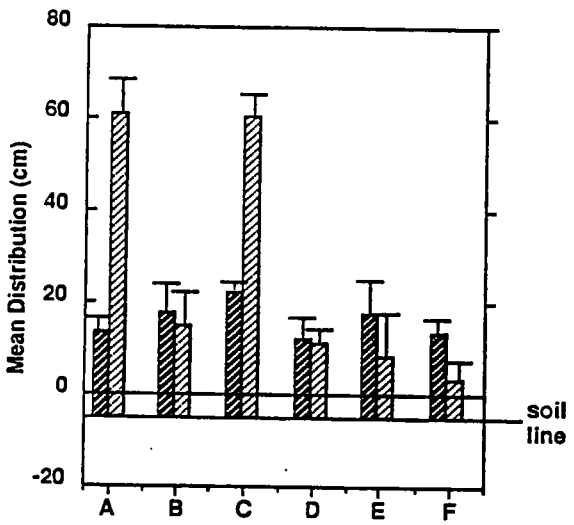

Figure 1C. Distribution of bacterial isolates from point of inoculation in sapwood tissue of container-grown red oak trees. Average tree height from soil line was 74.33 cm. A) *B. pumulis* 1-1; B) *P. denitrificans* 1-15; C) *B. pumulis* 3-23; D) *P. putida* 5-48; E) *B. alvei* 6-27; and F) *E. herbicola* 9-16. ▨ - Day 0; ▨ - Week 4. Inoculum was 10^8 cfu/ml. Bars represent standard error of the mean.

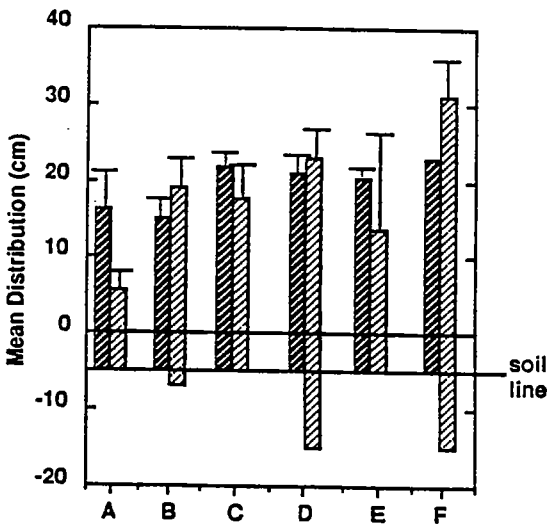

Figure 1D. Distribution of bacterial isolates from point of inoculation in sapwood tissue of container-grown live oak trees. Average tree height from soil line was 97.94 cm. A) *B. pumulis* 1-1; B) *P. denitrificans* 1-15; C) *B. pumulis* 3-23; D) *P. putida* 5-48; E) *B. alvei* 6-27; and F) *E. herbicola* 9-16. ▨ - Day 0; ▨ - Week 4. Inoculum was 10^8 cfu/ml. Bars represent standard error of the mean.

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Table 2. Evaluation of prophylactic treatment of container-grown live oak trees to challenge by *C. fagacearum*.

Isolate (Designation)	Treatment cfu/ml[1]	No. of Trees Treated		No. of Trees Diseased[2]		Crown Loss(%)[2]	
		Exp. I	II	Exp. I	II	Exp. I	II
P. denitrificans (1-15)	10^8	10	10	4a	10a	25a	68a
P. putida (5-48)	10^8	10	10	8b	10a	52a	51a
Control	Buffer	10	10	9b	10a	42a	90b

[1] Trees were injected with 1 ml of bacterial suspension or phosphate buffer 1 week before injection with 1 ml of a conidial suspension of *C. fagacearum* at 10^6 spores/ml.

[2] The means shown in each column for each of the experiments followed by the same letter are not significantly different (P>0.05) as determined by Duncan's multiple range test.

the control treatment and each of the prophylactically-treated trees became diseased. Red oaks did not appear to be protected from disease development by pretreatment with either of the isolates tested. This may be because of the high susceptibility of red oak to the oak wilt pathogen.

Studies in Mature Live Oak Trees

Studies were conducted to evaluate colonization and survival of selected bacterial isolates (1-15, 1-1, and Miss Y-20) in naturally grown live oaks and to establish the efficacy of the selected bacterial strains to control oak wilt under natural conditions. The three isolates were individually injected into mature trees in five different plots. Samples were taken at various times up to one yr after injection to determine the distribution and persistence of the introduced isolates. Sampling of the mature trees showed that isolate 1-15 (*P. denitrificans*) was recovered more consistently than the other isolates. Isolate 1-15 was found in the roots at three wk after treatment just below the point of injection and was isolated from the trunk at one wk and one yr after treatment and in the crown at one mo after treatment. Isolates Miss Y-20 and 1-1, were found only two ft above the point of injection at three mo and 1 wk after treatment, respectively.

As previously stated, three of the five plots were on the outer edge of an actively-progressing disease center. Over the course of 15 mo, 11 of 35 untreated and three of four treated trees in plot 8.5 became diseased. In plot 7.5A, 12 of 41 untreated and two of three treated trees became diseased, whereas in plot 6A all treated trees became diseased and 10 of 32 untreated trees became diseased. Results on plots 8A-1 and 8A are still pending since the plots are presently being challenged.

Natural challenge of the plots by *C. fagacearum* has and is taking place and tree survival appears to be low. The low survival of treated trees combined with poor recovery of the introduced isolates indicates that changes in experimental design to allow for improved introduction and survival of the bacterial isolates are necessary. For example, an improved delivery might allow for better distribution of the bacterium in the vessels where the pathogen is active.

Our results indicate that the introduced isolates probably survived in small pockets, or niches, throughout the tree. The method of initial delivery may have resulted in poor distribution within the tree.

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The bacteria may not have been introduced into some areas that were sampled, or once introduced, their populations declined below the level of our detection. Additionally, this study also suggests that criteria other than colonization and pathogen inhibition should be considered in the development of a biocontrol agent. For example, the ability of the potential biocontrol agent to survive at low water potentials may be an important factor. Scheffer et al. (1989) showed that an isolate adapted to a low water potential (-5.7 MPa) was able to establish itself better than the parent strain within an elm.

Affects of FIC on Spore Germination

The *P. denitrificans* isolate (1-15) was chosen for more detailed study of the mechanisms presumed to be important for biocontrol. As previously indicated, isolate 1-15 produced a large zone of inhibition on PDA plates when bioassayed with *C. fagacearum* conidia. The FIC produced by isolate 1-15 appeared to be highly fungitoxic since no evidence of growth was observed within the zone of inhibition even after two wk of incubation.

An FIC was partially purified by the protocol previously described. A regression analysis of germination probability of *C. fagacearum* spores exposed to the partially purified FIC indicated a LD_{50} of 1.2 units/ml ($P \leq 0.05$). The FIC significantly inhibited the germination of *C. fagacearum* conidia *in vitro*. Preliminary studies indicate that the FIC has no phytotoxic properties and it is assumed that this compound can be produced, and is active, in the xylem tissues of live oaks. Inhibitory effects toward other *Ceratocystis* spp. and unrelated tree fungi make isolate 1-15 and/or its FIC a potential biocontrol agent for other plant pathogens. There is a great deal more that can be learned about the FIC. For example, the chemical structure and composition of the compound(s) are important properties that should be investigated. These studies could lead to a biologically-derived, chemical control for oak wilt.

CONCLUSIONS

Currently there exists no biocontrol agent that consistently controls a vascular pathogen of trees. Although good results have been achieved with *in vitro* tests for potential biocontrol agents (O'Brien et al. 1984, Scheffer 1983, Tainter and Gubler 1973), none of the currently available systems significantly reduces mortality of mature trees. Oak wilt in live oaks presents a good opportunity for developing a biocontrol for vascular wilts of trees, because of the predictability of pathogen spread and disease progress in previously healthy trees. However, in order to develop a successful biocontrol for oak wilt, more needs to be known about microbial ecology in the vascular system of trees and how both abiotic and biotic factors may affect the establishment and effectiveness of a potential biocontrol agent.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the Texas Agricultural Experiment Station, Texas A&M University, and the USDA Forest Service, Southern Forest Experiment Station, for their support of this study.

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Protein-Based Method of Diagnosis/Prognosis of Oak Wilt Disease

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Xylem fluid samples were obtained from prepared branches of live oaks (*Quercus fusiformis*, *Q. virginiana*) and red oaks (*Q. texana*) which were classified as symptomatic or asymptomatic for oak wilt disease caused by the fungus *Ceratocystis fagacearum*. The xylem fluid was obtained by a vacuum flush method using 0.1% sodium dodecyl sulfate (SDS). The samples were concentrated and examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). A 35-kilodalton (kDa) polypeptide was detected in xylem fluid preparations from symptomatic but not asymptomatic trees. A 95% correlation was estimated between the presence of the 35-kDa band in SDS-PAGE gel separations of xylem fluid samples and the appearance of oak wilt symptoms. This estimate was based on sampling 59 oaks from three counties in central Texas. The 35-kDa polypeptide was also detected in live oak seedlings which were artificially infected with *C. fagacearum*. The gel-isolated 35-kDa fraction has been used to immunize guinea pigs for production of specific polyclonal antibody. The latter will be used to further test the feasibility of using the 35-kDa polypeptide in diagnosis and/or prognosis of oak wilt disease.

INTRODUCTION

Oak wilt is a systemic disease caused by the ascomycetous fungus, *Ceratocystis fagacearum* (Bretz) Hunt. No cure for this disease is yet available and the loss of live oaks (*Quercus fusiformis* Small and *Q. virginiana* Mill.) and Spanish oaks (*Q. texana* Buckl.) in central Texas has reached epidemic proportions. As of February 1992, oak trees in 46 central Texas counties were confirmed to harbor the pathogen (Texas Forest Service 1992). Only 31 counties reported the occurrence of oak wilt in 1985 (Appel et al. 1985). The legacy of devastation to the American elm population in the United States and Canada, caused by a related systemic pathogen *Ophiostoma* (*Ceratocystis*) *ulmi* (Pomerleau 1970), is vivid in our memory and has served as a catalyst to accelerate research on early diagnosis and treatment of oak wilt.

Recognition of oak wilt depends on appearance of some or all of the following five visible symptoms (Appel 1986); 1) chlorosis and necrosis of the midvein and smaller lateral veins (live oaks only), 2) necrotic scorch of leaf tips and margins, 3) chlorosis and necrosis of the interveinal regions of leaves (live oaks only),.4) browning and bronzing of leaf margins, necrosis of entire leaf, and wilting (deciduous Spanish oaks and shin oaks (*Q. sinuata* var. *breviloba* (Torr.) C. H. Mull.), and 5) general, non-specific chlorotic and necrotic mottling of leaves and associated crown die back. Fungal mats are frequently detected under the bark of symptomatic Spanish oaks. The fungus can be cultured and identified as *C. fagacearum* and the fungal mat thus confirms the diagnosis of oak wilt disease in these trees. Fungal mats, on the other

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hand, are not found in diseased live oaks. Direct isolation of the pathogen from symptomatic live oaks is a time-consuming procedure. Tissue samples obtained from branches (> 1 in. diam.) or from sapwood of the trunk (*i.e.*, bole samples) have been cultured on acidified potato dextrose agar at room temperature (Appel 1986). The presence of *C. fagacearum* was correlated with symptoms of oak wilt in 38% of trees when bole samples were cultured, and only 19% for branch tissue samples.

These results reflect several problems associated with current research efforts on diagnosis and treatment of oak wilt disease. Diagnosis based on visible symptoms and/or isolation of the pathogen is not totally reliable. Although the frequency of isolation of *C. fagacearum* from live oaks with both symptoms (1) and (2) listed above is approximately 50% (Appel 1986), these diagnostic features provide little information about the duration of the fungal infection in the tree, extent of the tissue infection, or prognosis and potential for recovery if fungicidal treatment was initiated. Ideally, a diagnostic and prognostic indicator(s) is (are) needed which will allow the field researcher to detect infection by *C. fagacearum* at the earliest possible time post-infection before the characteristic leaf symptoms are visible. This would permit identification of candidate trees for early administration of fungicides and probably increase the efficacy of the antifungal agent.

It should be emphasized that symptoms often appear after the systemic fungal infection is well established. Prophylactic treatment of oaks in the endemic areas would be exorbitantly expensive at this time and may not be effective even if implemented. Early diagnosis and coordinated fungicidal treatment of infected trees is a rational approach to the problem. The ability to provide an accurate and sequential prognosis of infected trees which undergo a course of treatment with experimental fungicides is as important as early diagnosis. At present, it is not possible to precisely monitor response of infected trees to systemic administration of experimental fungicides.

In this paper we present preliminary results of our attempts to identify a molecular indicator of *C. fagacearum* infection of live oaks and Spanish oaks. We have focused on a 35-kilodalton (kDa) polypeptide which has been detected in xylem fluid of infected trees. We provide evidence for the possible application of this polypeptide to diagnosis/prognosis of oak wilt based on a 95% correlation between occurrence of leaf symptoms of the disease and presence of the 35-kDa polypeptide as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

METHODS

Collection and Preparation of Tissue Samples from Symptomatic and Asymptomatic Trees

Samples of xylem fluid were collected from live oaks and red oaks within and outside infection centers in Travis, Bandera, and Williamson Counties of central Texas. The exact sites of symptomatic trees from which samples were obtained are described in Table 1. These sites were chosen because they had been identified by other investigators as established infection centers, they were scattered through parts of three counties in Texas, and the majority of trees sampled had not been treated with propiconazole (Alamo®). Asymptomatic trees were chosen on the basis of their distance from known infection centers (1-5 mi) or, if in an infection center, the complete absence of any leaf symptoms or canopy loss (Table 2). The accession number(s) or species of oak in parentheses after the name of the site indicates the representative sample(s) examined in Figures 2, 3, 6, and 7.

Symptoms Used for Identification of Oak Wilt

Selection of symptomatic live oaks for research was based on the presence of leaves attached to the tree showing a pattern of necrosis along the mid-vein extending to the lateral veins with the remainder of the leaf being green. This pattern is called veinal necrosis and is the most reliable symptom of the presence of infection by *C. fagacearum* (Johnson and Appel 1989). The leaf tissue directly adjacent to the mid- and lateral veins may also appear chlorotic (loss of chlorophyll). Canopy loss or defoliation of infected trees occurs in a characteristic pattern and was also used to select symptomatic trees within a population if the leaves of the trees did not clearly show veinal necrosis. The combination of veinal necrosis, chlorosis, and percent canopy loss provided

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Table 1. Sites of sampling symptomatic trees (live oaks and red oaks) in central Texas.

Greater Austin		
MLK	=	Martin Luther King; trees on the north side of MLK at Speedway (49-52, 120)
DE	=	Deep Eddy site; trees on the north side of Lake Austin Blvd. at Hearn St.
LL	=	Las Lomas site; trees on the east side of Rollingwood at Las Lomas St. (64, 118, 122)
SEU	=	St. Edward's University campus; trees on the north side of University Ave.
Georgetown		
AB	=	Alice Briggs Farm; trees on farm off Hwy. 71 near County Road 56, northeast of Georgetown (47, 119)
Round Rock		
RR	=	Robinson Ranch; trees on ranch 1/4 mi north of Parmer Lane at Hwy. 620N
Medina		
MJ	=	Milton Johnson Ranch; trees on ranch off Hwy. 16, 2 mi east of Medina (red oak)
TC	=	Tommy Curbo Ranch; trees on ranch off Hwy. 337, approximately 3 mi southwest of Medina (red oak)
JAG	=	James Arthur Gallant Ranch; trees on ranch off Hwy. 337, 4 mi southwest of Medina
KS	=	King Stokes Ranch; trees on ranch off Hwy. 16, north of Medina
ST	=	Susan Tracy Ranch; trees on ranch off Hwy. 337, approximately 6 mi southwest of Medina
JF	=	J. Faglie Factory site; trees off Hwy. 16, north of Medina
JH	=	J. Harvey Ranch; trees on ranch off Hwy. 337, 4 mi southwest of Medina

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Table 2. Sites of sampling asymptomatic trees (live oaks and red oaks) in central Texas.

Greater Austin
- MLK = As above (91)
- DE = As above
- MC = Matador Circle; trees off Toro Canyon Dr. one mile from nearest site of an infection center (56-58)

Round Rock
- RR = Robinson Ranch, trees on ranch 1/4 mile north of Parmer Lane at Hwy. 620N; trees in an isolated motte apart from the infection center
- TC = As above (red oak)

the means for rating the severity of symptoms in individual trees (Figure 4).

Selection of symptomatic red oaks for our study was based on the presence of wilted, bronze-tipped leaves (i.e., tip burn) or brown, dead leaves (Johnson and Appel 1989) which occurred along the entire length of branches rather than only at the end of a branch. Symptom severity was based on percentage of canopy that was wilted or leaves that showed tip burn or were totally brown.

Collection of Branches from Symptomatic and Asymptomatic Trees

Branches with diameters between 1/4 and 3/4 of an inch were cut with pruning shears sterilized with 70% ethanol. In the case of symptomatic trees, branches were cut from the sides of the tree with symptomatic leaves at 8-15 ft above ground level. All leaves and lateral branches smaller than 1/4 in. in diameter were removed and the remaining branch was cut into 10 in. segments. These segments were labeled to show orientation of apex and base of the branch (i.e., to indicate direction of xylem fluid transport). The segments were bundled, bagged, labeled according to the tree from which they were taken, and placed on ice. They were stored in the laboratory at 4°C until prepared for flushing. All wounds to the experimental trees were completely sealed with Treekote®.

Preparation of Branches for Xylem Tissue Flush

A modification of the method reported by Gregory (1966) was employed (Figure 1). All dissection instruments were surface sterilized with 95% ethanol and flamed. All glass apparatuses were acid cleaned and thoroughly rinsed with distilled water. All plastic or rubber equipment was cleaned overnight with 10% sodium hypochlorite and thoroughly rinsed with distilled water.

The bark, phloem, and cambium from each branch segment were removed. Each end of the segments was cut diagonally to increase surface area. The branches were sealed in the apparatus shown in Figure 1 using Parafilm and a rubber stopper. The branch was oriented so that the 0.1% SDS in distilled water (4°C), which was used to flush the xylem tissue, would flow in the direction of natural xylem fluid movement. A plastic funnel was sealed to the top of the branch segment which extended above the rubber stopper. Branch, stopper, and funnel were then placed in a 500 ml sidearm flask linked to a trap and vacuum pump.

Collection and Concentration of Samples

Between 20 and 40 ml of 0.1% SDS were poured into the funnel and vacuum was applied until all the SDS had flushed through the segment. The maximum potential of the pump was 30 in. Hg. The time required for 1 ml of 0.1% SDS to pass through

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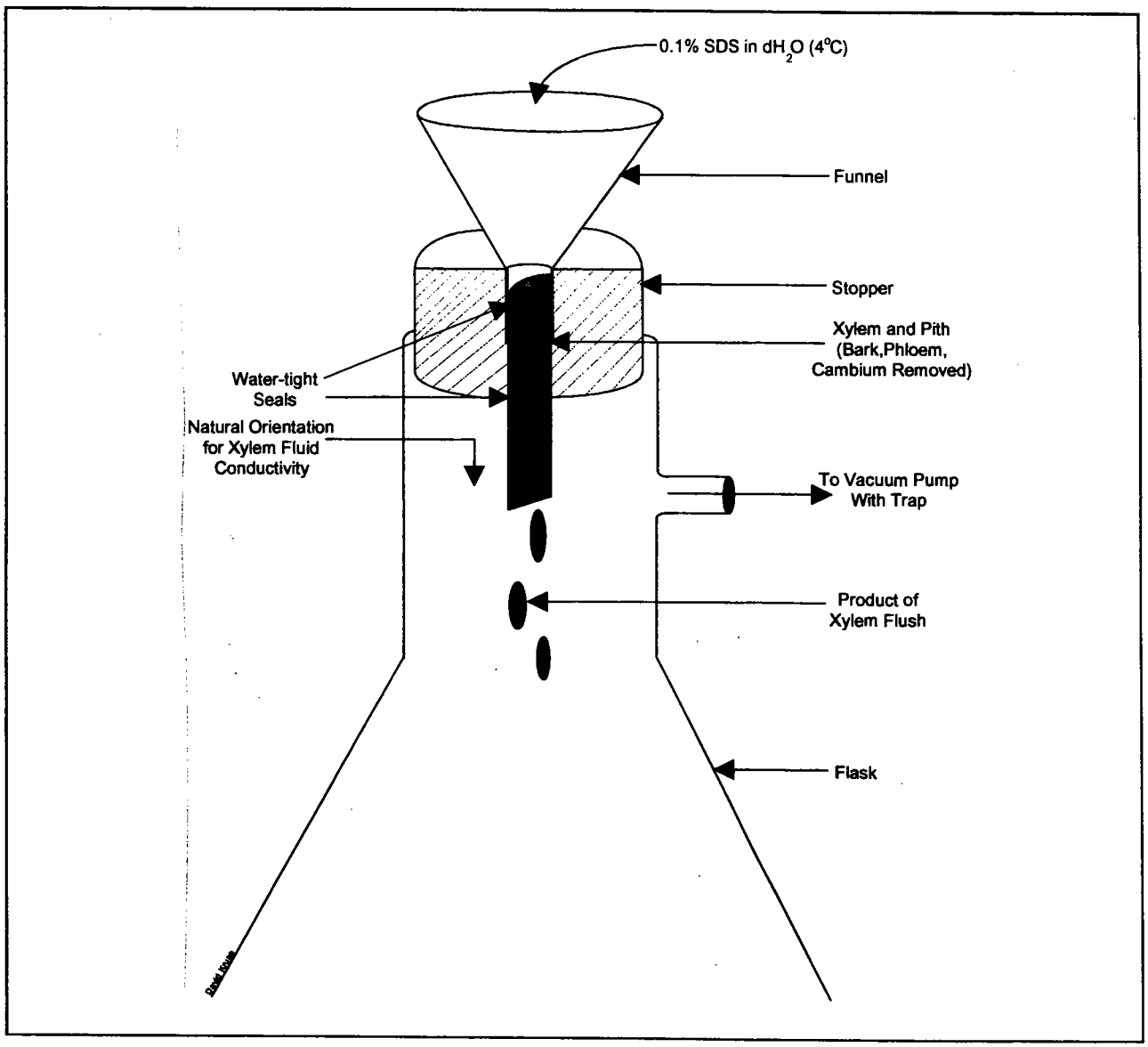
Figure 1. Diagram of apparatus used to collect xylem fluid from branches of oak trees and stems of live oak seedlings.



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a 3 in. branch segment (approx. 1/8 in. diam) obtained from an asymptomatic live oak was 2-3 min at maximum vacuum. The time required for the same volume of fluid to pass through a branch segment from a symptomatic tree under identical conditions was 12 min. Products of 3-5 segments of the same branch were flushed in this manner and 500 µl of product was plated on acidified PDA and incubated (25°C, seven dys.) for detection of *C. fagacearum*. The remaining product of each flush was filtered through a 0.2 µm cellulose nitrate membrane (Millipore) in a Millipore Swinnex system. The filtrate was then dialyzed (6,000-8,000 MW cutoff; Biodesign, Inc.) against distilled water (4°C) for 72 hr (8 changes of dH_2O) to remove the SDS. The retentate was then lyophilized, rehydrated with 2 ml Milli-Q distilled water, aliquotted into Eppendorf centrifuge tubes, and reconcentrated by rotary evaporation. The concentrated samples were then stored at -70°C until analyzed by SDS-PAGE.

SDS-PAGE

Electrophoresis was conducted with a 15% slab gel and the discontinuous buffer system described by Laemmli (1970). Concentrated samples were dissolved in buffer that contained 2.3% SDS, 0.4 M 2-mercaptoethanol, 0.002% bromophenol blue, 5.0% glycerol, and 62.5 mM Tris hydrochloride (pH 6.8). The samples were boiled for 3 min. Approximately 20 µg of protein (Jones et al. 1989) of each sample preparation was applied to the gel. Electrophoresis was performed at constant current (20 mA) at room temperature for 1.5 hr. The polypeptide bands were revealed by the silver stain method of Merril et al. (1984). Protein standards used included the Rainbow markers (Amersham), with a molecular weight (MW) range of 14.3 to 200 kDa, and the medium range standards from Bio-Rad with MW of 13.3-97.4 kDa.

Experimental Infection of Live Oak Seedlings

One-yr-old seedlings (12-24 in. high) were grown from acorns provided by Mortellaro's Nursery, San Antonio, and Turkey Creek Nursey, Round Rock, Texas. The seedlings were incubated in growth chambers at 25°C with 12 hr light/dark photoperiod. The stem of each seedling was inoculated with *C. fagacearum* at about 1.5 in. above ground level. The surface of the stem was first washed with 70% ethanol. A 23-gauge hypodermic needle was used to puncture the bark and open a wound into the xylem tissue. Approximately 2.5 x 10^6 conidia of strain TR847 (provided by D. Appel) were suspended in 0.9% sterile saline and inoculated into the xylem tissue. Control seedlings were inoculated with saline alone. Seedlings were sacrificed two wk to two mo post-inoculation. The stem between the inoculation site and 12 in. above this site was removed and subdivided into four in. segments. The preparation of xylem tissue for flushing with 0.1% SDS, sample collection and concentration were conducted as described above for tree samples collected from the field.

Histology and Immunofluorescence

Histological preparation of xylem tissue from infected seedlings was performed as previously described (Cole et al. 1988), except that osmium tetroxide staining was omitted. Thick sections (approx. 1 µm) of infected tissue were attached to glass slides and prepared for examination by immunofluorescence for the presence of *C. fagacearum*. Antiserum was raised in rabbits against an alkali soluble-water soluble (ASWS) extract of the isolated mycelial wall of *C. fagacearum* by the method used for other fungal pathogens (Cole and Sun 1985, Cox and Britt 1985). New Zealand white rabbits (two females, 4 kg each) were immunized with the ASWS antigen as reported (Cole et al. 1987). The antiserum, diluted 1:50 in 50 mM Tris-HCl buffer (pH 8.0), was incubated with the sections of infected tissue followed by buffer washes and incubation with goat anti-rabbit immunoglobulin G fluorescein isothiocyanate (FITC) conjugate (Sigma) as described (Cole et al. 1987). The sections were again washed in buffer and then examined by immunofluorescence microscopy. Sections reacted with preimmune rabbit serum followed by the secondary antibody/FITC conjugate, or FITC alone served as controls.

Preparation of 35-kDa Antigen for Antiserum Production

Xylem fluid samples from four symptomatic live oaks with clearly resolved 35-kDa fractions (accession nos. 118, 119, 120, 122), as detected by SDS-PAGE, were

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Figure 2. SDS-PAGE gel separations of xylem flush preparations from symptomatic (Sy) and asymptomatic (As) live oaks.

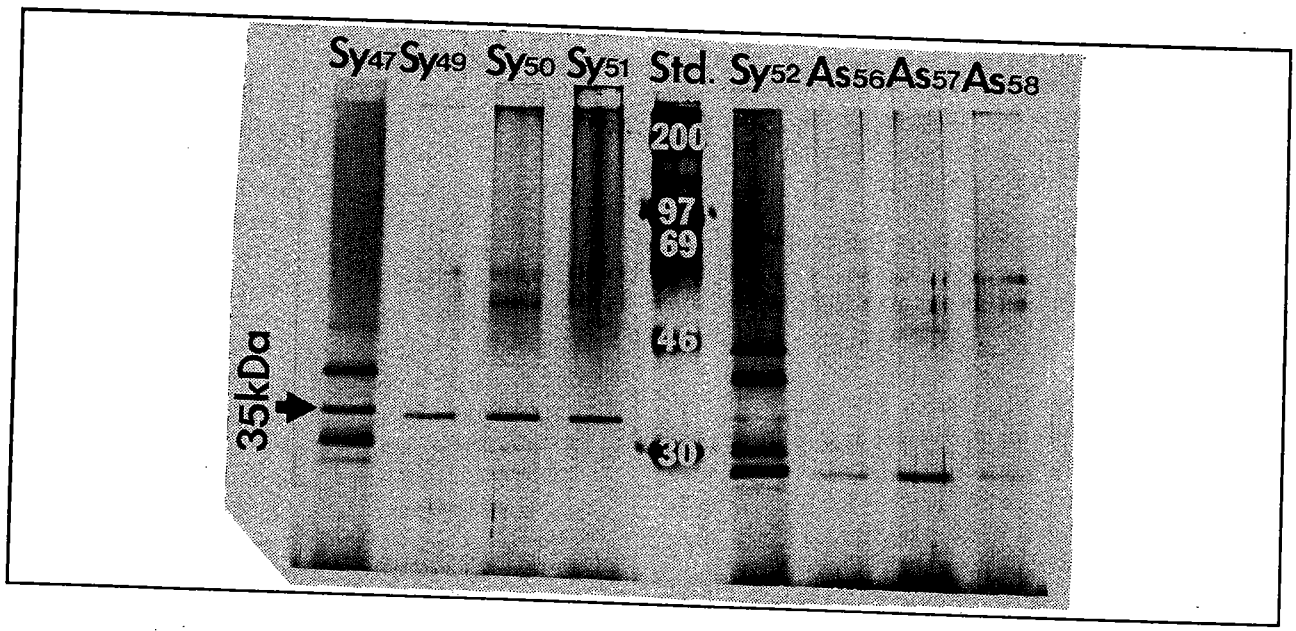
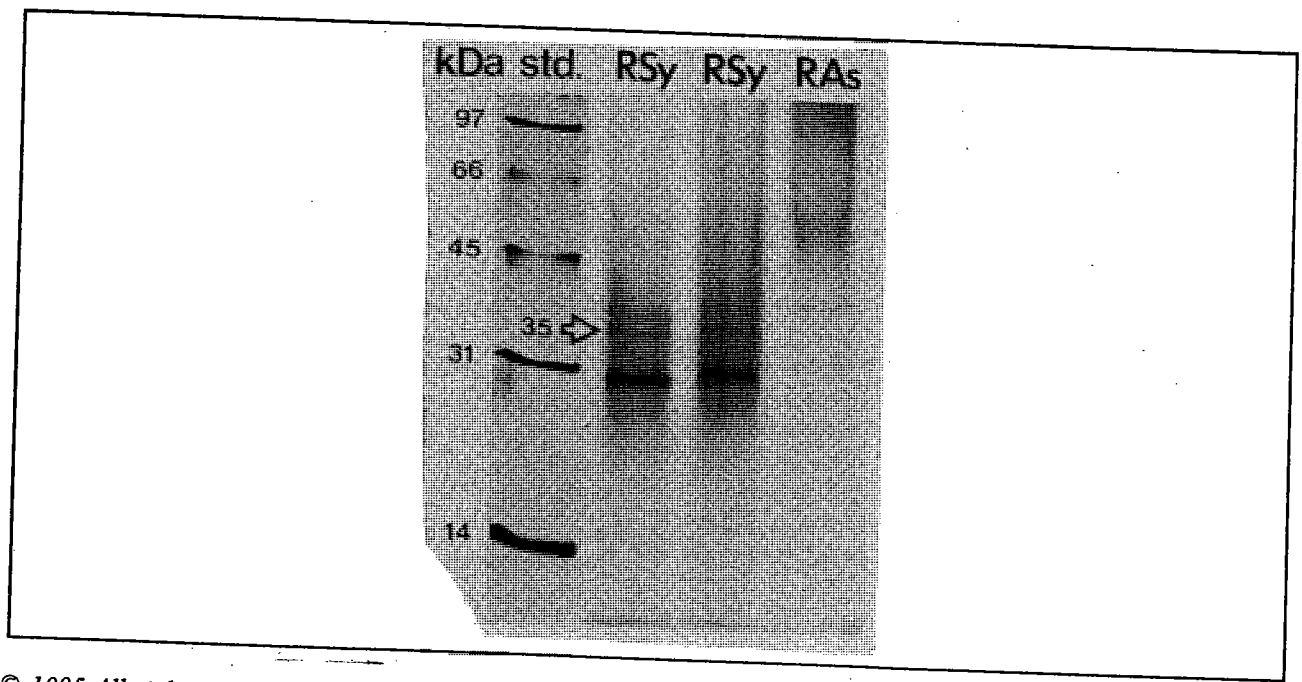


Figure 3. SDS-PAGE gel separations of xylem flush preparations from symptomatic (RSy) and asymptomatic (RAs) red oaks.



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pooled and concentrated by rotary evaporation. The pooled concentrate (approx. 50 µg of protein) was subjected to SDS-PAGE. The concentrated 35-kDa band has been excised from the gel, the polypeptide has subsequently been isolated from the gel slice by electroelution as reported (Cole et al. 1989), and the antigen has been used to immunize guinea pigs for production of anti-35 kDa polyclonal antibody (Kruse and Cole 1992) for future studies.

RESULTS AND DISCUSSION

Detection of a 35-kDa Polypeptide in Symptomatic Oaks

The results of a representative SDS-PAGE gel of xylem fluid samples obtained from symptomatic (Sy 47, 49-52) and asymptomatic live oaks (As 56-58) are shown in Figure 2. The 35-kDa band is well defined in the concentrated fluid preparations from the symptomatic trees and appears to be a single macromolecule based on its silver-staining characteristics. No silver-stained 35-kDa band was visible in concentrated xylem fluid preparations from asymptomatic trees. Difference in intensity of the 35-kDa band is evident in samples Sy 47 and Sy 52 in Figure 2. We attempted to apply the same amount of protein (approx. 20 µg) to each lane of the gel. Note that the adjacent band (approx. 40 kDa) above the 35-kDa fraction is about the same intensity in these two samples. The amount of the specific 35-kDa polypeptide in Sy 52 appears to be significantly less than that shown in Sy 47. The former was a xylem fluid sample obtained from a live oak which demonstrated early symptoms of disease (very few leaves with veinal necrosis/chlorosis and little canopy loss). Sy 47, on the other hand, demonstrated more extensively developed symptoms of oak wilt (see Figure 4).

These data suggest that the initial appearance of the 35-kDa polypeptide in xylem fluid may correlate with early *C. fagacearum* infection. A more sensitive and specific assay than SDS-PAGE is required to test this hypothesis. As indicated above, we have already initiated work to generate specific antibody against the purified 35-kDa fraction which will be used in enzyme-linked immunosorbent assays of xylem fluid samples (Ausubel et al. 1989).

The 35-kDa band also was well defined in symptomatic red (Spanish) oaks (RSy) but absent from asymptomatic (RAs) trees (Figure 3). The duration between initial infection, early leaf symptoms, and canopy loss is much shorter in red oaks than in live oaks. It may be more efficient and accurate, therefore, to monitor changes in levels of the 35-kDa polypeptide in infected red oaks during progressive stages of oak wilt disease than in live oaks. Such studies should be performed on infected trees which are untreated or treated with experimental fungicides. We recognize the possibility at this stage that the 35-kDa polypeptide could be more useful for forecasting the probable course and termination of wilt disease in oak trees than for early diagnosis of infection. Detection of changing levels of the 35-kDa polypeptide concentration by the ELISA may be a valid prognosis of oak wilt and a useful indicator of the efficacy of systemic fungicidal treatment of *C. fagacearum*-infected trees.

The results of our studies of 59 trees from 13 different infection centers and selected mottes which do not apparently harbor oak wilt are presented in Figure 4. Xylem fluid samples from one live oak and one red oak revealed the 35-kDa polypeptide but showed no leaf symptoms or canopy loss at the time of sampling. Both trees were adjacent to infected stands. One month after sampling, oak wilt symptoms appeared. Bole tissue samples were plated on acidified PDA and showed the presence of *C. fagacearum*. Our detection of the 35-kDa band in SDS-PAGE gel separations of xylem fluid from these presymptomatic trees is evidence for the diagnostic potential of our 35-kDa polypeptide detection method.

The absence of the 35-kDa band from xylem fluid of three live oaks with symptoms of advanced oak wilt cannot be explained at this stage in our study. It is possible that these trees received systemic fungicidal treatment without our knowledge. It is also possible that our xylem flush preparations from these trees were not sufficiently concentrated. In spite of these recognized false negative results, our preliminary results show ≥ 95% correlation between presence or absence of the 35-kDa polypeptide band

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Figure 4. Scatter plot showing correlation between presence/absence of the 35-kDa polypeptide and symptomatic/asymptomatic live oaks and red oaks.

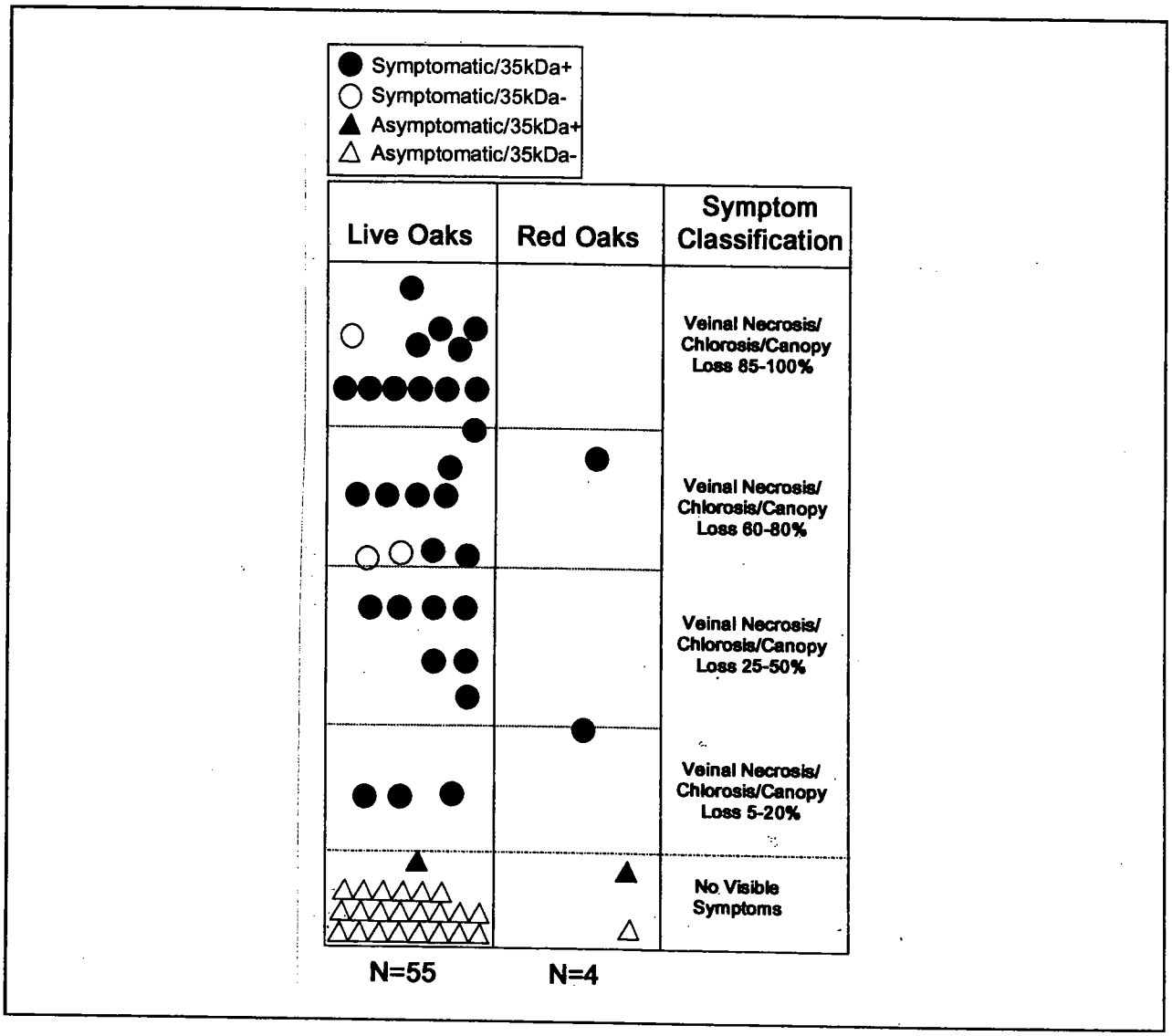

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Figure 5. Immunofluorescence of *C. fagacearum* using anti-cell wall/FITC conjugate (A) and control serum (B). Presence of *C. fagacearum* hyphae (arrows) in vessels of an infected live oak seedling is shown in (C). Bars represent 15 μm.

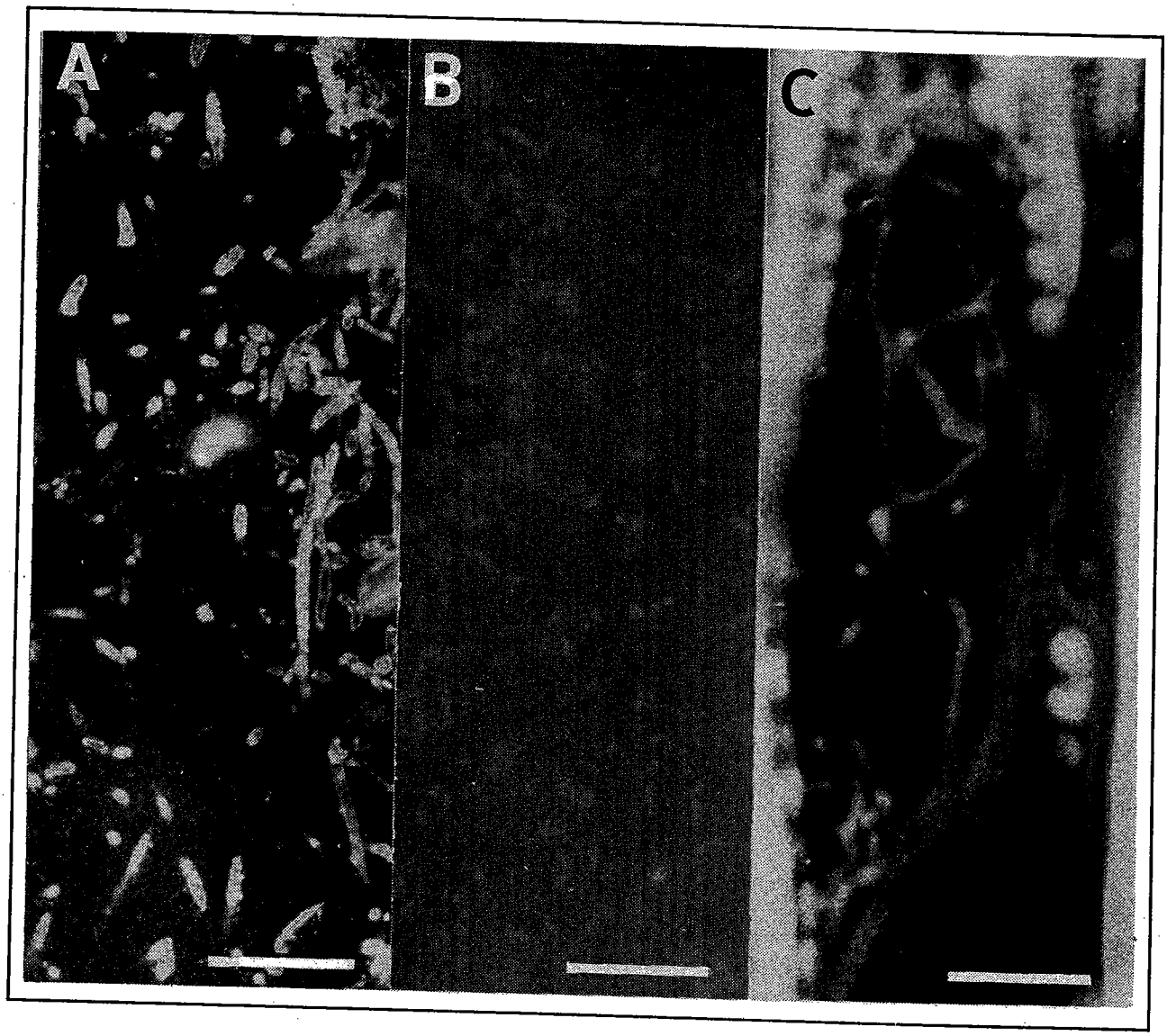

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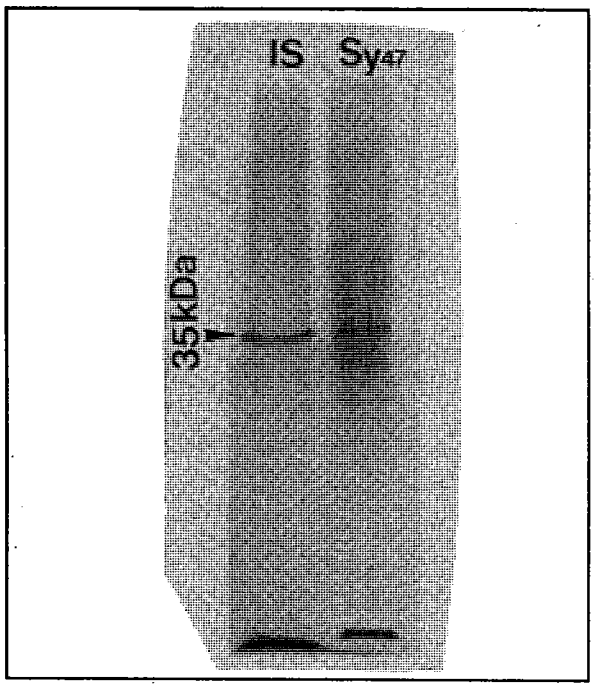
Figure 6. SDS-PAGE gel separations of xylem flush preparations from an infected seedling (IS) and symptomatic live oak (Sy 47).

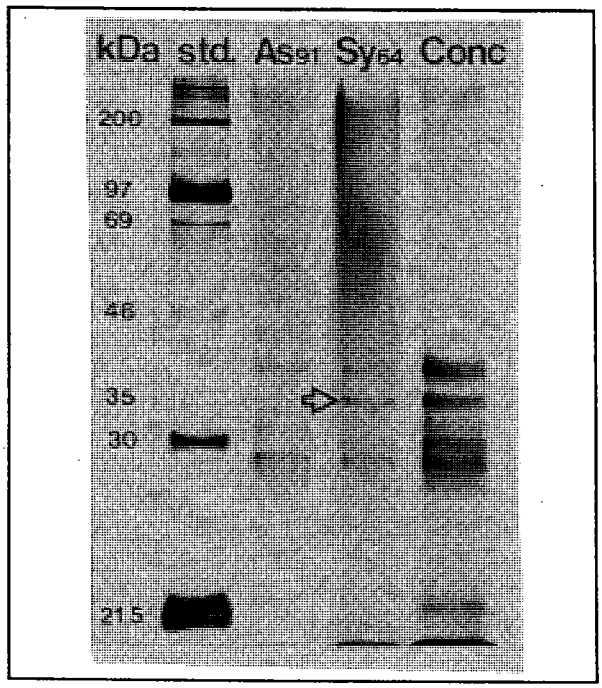


by SDS-PAGE in xylem fluid flushes and the appearance or absence of oak wilt symptoms, respectively.

Infection of Live Oak Seedlings

The antiserum raised in rabbits against the ASWS wall fraction of *C. fagacearum* showed good sensitivity and specificity. In Figure 5A, the anti-*C. fagacearum*/FITC conjugate has reacted with hyphal and conidial elements. In the control reaction (Figure 5B), the preimmune rabbit serum was incubated with sections of *C. fagacearum* followed by incubation with goat anti-rabbit/FITC conjugate. Essentially no binding of primary or secondary antibody to the hyphae and conidia was evident.

Figure 7. SDS-PAGE gel separations of xylem flush preparations from an asymptomatic (As) and symptomatic (Sy) live oak, and separation of a pooled and concentrated preparation from four symptomatic live oaks (Conc.).



In Figure 5C, the anti-*C. fagacearum*/FITC conjugate was reacted with xylem tissue from an infected live oak seedling. The seedling was sacrificed at two wk post-inoculation with *C. fagacearum*. Hyphal elements (arrows) were visible within a vessel. The nonspecific fluorescence is due to FITC binding to wall components of the host cells. The results of this study demonstrate that colonization of xylem tissue (at least vessel elements) by *C. fagacearum* hyphae does occur in experimental seedlings. Live oak seedlings may, therefore, be useful to monitor the appearance of the 35-kDa polypeptide in xylem fluid preparations.

In Figure 6, a 35-kDa band is faintly visible in the SDS-PAGE gel separation of the xylem flush preparation of an infected seedling (IS). The seedling was sacrificed two wk after inoculation. The adjacent

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lane (Sy 47) shows a corresponding 35-kDa band in the electrophoretically separated xylem flush preparation from an infected live oak. The results of our seedling studies are still ambiguous. Most of the infected seedlings tested did not show the 35-kDa band in their xylem fluid preparations. None of the non-infected seedlings showed the fraction. These data suggest that the 35-kDa polypeptide is not a product of *C. fagacearum*, but instead may be a response protein released by the host. Our ability to test this hypothesis awaits the production of specific antiserum against the 35-kDa polypeptide.

Isolation of the 35-kDa Antigen

We have pooled xylem flushes from symptomatic live oaks in an attempt to concentrate the 35-kDa fraction. The results of this procedure are shown in Figure 7 (lane Conc.). Adjacent preparations from symptomatic (Sy 64) and asymptomatic live oaks (As 91) also are shown. We have isolated the 35-kDa band from the pooled preparation (solid arrow in Figure 7) by gel excision followed by electroelution (Cole et al. 1989). The concentrated polypeptide (approx. 6.5 µg/gel) has been used to immunize guinea pigs for production of polyclonal antibody. This reagent will be used in Western blots (Ausubel et al. 1989) to determine whether the 35-kDa polypeptide can be detected in cell wall and cytosol preparations of *C. fagacearum* grown *in vitro*. The anti-35-kDa serum will also be used in the ELISA to continue our survey of symptomatic and asymptomatic oak trees for the presence of the specific polypeptide. As a further extension of these studies, we will produce and test monoclonal antibodies raised against the 35-kDa polypeptide. The application of these reagents to the ELISAs of xylem fluid preparations can significantly increase the sensitivity and specificity of the assays.

CONCLUSIONS

1. A 35-kDa polypeptide has been identified in xylem tissue flushes of symptomatic live oaks, Spanish oaks, and artificially-infected live oak seedlings.

2. A 95% correlation exists between the presence of the 35-kDa polypeptide and symptoms of oak wilt.

3. Production of antiserum against the 35-kDa polypeptide is in progress. Anti-35-kDa polyclonal antibody will be used to determine the source of the 35-kDa fraction by Western blot analysis, and for application in the ELISA for testing sensitivity/specificity of the immunological reagent in diagnosis and prognosis of oak wilt disease.

ACKNOWLEDGMENTS

The authors are grateful to Mortellaro's Nursery and Turkey Creek Nursery for their kind contribution of live oak seedlings to this project. Dr. K. R. Seshan provided assistance with the immunofluorescence preparations. This work was supported by a research grant from the Lower Colorado River Authority to G. T. Cole.

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Section III
State Oak Wilt Management Programs Outside Texas

Oak Wilt Management in Michigan

Johann N. Bruhn

Michigan is initiating an oak wilt management program which stresses detection and containment of existing foci, and prevention of new focus establishment. Public involvement will be central. Systematic aerial surveillance is essential for efficient detection. Early detection leads to treatment of smaller foci, which minimizes site disturbance, mat production, and tree loss. Foci are contained by separating grafted root systems along barrier lines using a five foot long vibratory plow blade (or Vapam®). Reference tables developed for local conditions are used for barrier placement. All living oaks inside the barrier are removed, and the resulting stumps are treated to prevent re-grafting. Focus establishment can be prevented by sanitary treatment of all trees which may produce spring-time mats, prevention of unsupervised movement of diseased wood, the avoidance of oak wounds in the spring and early summer, and the immediately treatment and dressing all such wounds.

INTRODUCTION

Oak wilt is a serious disease of oaks in Michigan. The causal fungus, *Ceratocystis fagacearum* (Bretz) Hunt, disrupts the vascular systems of oaks. Red oaks (subgenus Erythrobalanus) are especially susceptible to oak wilt, and are rapidly killed by the disease. Expanding pockets of dead trees called foci enlarge as a result of fungus dissemination through root grafts. New foci are established by insect vectors. The symptoms and much of the biology of oak wilt have been described previously (MacDonald and Hindal 1981, Sinclair et al. 1987). Although oak wilt and Dutch elm disease are very similar, the outlook for oak wilt control in Michigan is more positive, because overland spread of oak wilt is much less efficient.

Michigan's red oak resources are valuable. Red oaks generally have high landscape, wildlife habitat, and forest products values. Waddell et al. (1989) indicate that Michigan holds 3.4 billion board feet of select red oak and 593 million board feet of other red oak.

In Michigan, interest in controlling oak wilt has revived since 1980, due to heightened public concern, the perceived threat to the state's oak resources, a better understanding of oak wilt epidemiology, and the development of more efficient control methods. In 1991, an Inter-Agency Working Group led by the Michigan Departments of Agriculture (MDA) and Natural Resources (MDNR) was organized to formulate a Cooperative Oak Wilt Program for Michigan. University scientists and Cooperative Extension Service personnel are key players. A formal Oak Wilt Survey and Diagnosis Form (located at the end of this paper) has been designed for use by forestry professionals who have been specifically trained in oak wilt diagnosis, sampling, and reporting. Data from completed forms are entered into an oak wilt database housed with the MDA. The database tracks suspected sites, confirmed active foci, and confirmed but inactive foci. Periodic reports summarizing incidence by county will be made available to cooperators. Follow-up activities will include public education and information, and establishment of control and regulatory programs.

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as on roundwood with attached bark cut from colonized trees. Mats form during the year following tree death. In Michigan, numerous mats form in May and early June on trees that wilted during the previous July or early August. Trees that wilt in June or early July may produce mats that autumn. Trees that wilt in late August or September often fail to produce mats at all. Spring mat production is most responsible for overland disease spread (Juzwik et al. 1985), because most mats are produced in the spring, when insect vectors are most abundant, and trees are most vulnerable and easiest to wound in the spring.

Establishment of new foci depends on insect vectors. Sap-feeding beetles (family Nitidulidae) may be the only important insect vectors of oak wilt in Michigan. Oak wilt foci are initiated through fresh wounds, usually caused by storm damage or human activity. Wounds may remain effective as infection sites for as long as a week in the spring. However, beetles are attracted to a wide variety of other plant and animal debris as well. Also, insect transmission of oak wilt over distances greater than one mile is apparently rare (Juzwik 1983). As a result, many fresh wounds on oaks are never visited by insects carrying the fungus. Humans can help vector the fungus by moving diseased wood with attached bark to unaffected sites.

Focus Expansion (Root Graft Spread)

More than 95 percent of diseased trees in Minnesota appear to become infected via root graft transmission (Juzwik 1983). We believe the same is true in Michigan, yet very little is known about the extent of root grafting among trees. Studies of disease spread in red oak stands suggest that the likelihood of any two trees being root grafted is related to their diameters, the distance between them, and soil texture and depth. Local spread of oak wilt has been shown to occur over greater distances in shallow soils than in deeper soils (Gillespie and True 1959), and in sandy than in loamy soils (Bruhn et al. 1991, Gillespie and True 1959). Root systems of northern red oak (*Quercus rubra* L.) were found to extend farther in a loamy sand than in a fine sandy loam (Garin 1942). In Menominee County, Michigan, the greatest single annual distance we have attributed to root grafting is 40.9 ft. Other work suggests that root graft spread may occur over distances of 60 ft or more between large trees (Himelick and Fox 1961, Jones 1971), but this may take more than a single year.

COMPONENTS OF AN OAK WILT CONTROL PROGRAM FOR MICHIGAN

Public Awareness and Recognition

Public relations are crucial to program success. Land managers, woodsworkers, and property owners must be included in the program from its inception. This includes agencies and individuals using or moving oak roundwood, or pruning or injuring oaks. They need to recognize oak wilt and understand the ongoing control program. Oak wilt causes greater concern and value loss on homesites than in wildland settings. A large proportion of oak wilt foci occur on residential sites. This attests to both 1) the significance of the human elements in disease spread and establishment, and 2) the importance of public participation in an oak wilt control program. Awareness of the potential impacts of oak wilt motivates local property owner involvement. They are asked to report suspicious oak mortality to a designated office. The risk associated with transporting infected oak wood is made clear.

The spectacular nature of oak wilt in areas dominated by red oak generates its own publicity. Foci often begin with a single infected tree, but eradication of foci involves the sacrifice of "many" surrounding apparently healthy trees. The landscape disruption associated with necessary tree removals and emplacement of root graft barriers also must be considered. As painful as the cure may be, most property owners quickly relate to the alternative scenario of continual enlargement of foci, with eventual overland spread. The effectiveness of a thorough, carefully conceived control program has the potential for generating public support to an extent that few diseases share.

One or more designated persons should be available throughout the year to answer questions. Letters itemizing options available to the property owner, and authorizing control treatments, should be mailed (certified) by the appropriate agency well in advance of treatment to allow feedback. Public

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meetings are beneficial. Authority may be desirable for enforcement of control practices in rare cases where property owners with diseased trees prefer not to participate in the control program. This has been necessary only once in ten years in Menominee County, Michigan. Available authority varies among states, resting in Michigan with the state's Department of Agriculture. Where authority is initially lacking, it may be possible to establish appropriate local tree ordinances.

Detection

The cause of oak mortality in an area must be properly diagnosed. Once oak wilt has been diagnosed, the first step is to locate all foci. Systematic aerial photography is a great help in locating oak wilt foci. Color infrared transparencies taken in late July or early August, when most infected trees display symptoms, work very well. At a scale of 1:1,250, individual trees (and woods roads) are easily distinguished, permitting detection and location of new foci. Healthy trees appear bright red; sick trees appear pink; wilted trees appear tan; and leafless trees are black. During late July or early August, new foci will appear as one to several tan trees. Older foci are recognized for having one or more tan and/or pink trees surrounding a pocket of black trees or an opening.

Systematic aerial photography is superior to both aerial sketch mapping and ground-based searching wherever road access is minimal or poorly represented on available maps. High-resolution color video photographic systems have recently become available for systematic aerial surveillance. This type of system will be tested in Michigan, and may prove more useful, less expensive, and/or less cumbersome than photographic transparencies.

Focus Containment

Historically, various techniques have been employed in efforts to halt the root graft spread of oak wilt. Techniques such as clearing or poisoning a buffer strip of healthy trees around disease foci are often unsuccessful because the root systems of treated trees commonly survive. The living root systems of these "decapitated" trees may be grafted simultaneously to both diseased and healthy trees. When this occurs, the oak wilt fungus may be conducted across the buffer strip and into surrounding healthy oaks.

Correct placement of root graft barriers is critical for efficient disease control. The cost of root graft barrier construction is substantial, in terms of both direct expense and the sacrifice of apparently healthy trees left within barriers (especially on residential or park property). The strategy of root graft barrier placement is to contain the pathogen while sacrificing as few healthy trees as possible. Barrier locations are best determined between late July and mid-August, by which time most of the year's symptomatic trees have become evident. Since the exact underground position of the pathogen between diseased and healthy trees is uncertain, an attempt is made to include, inside a root graft barrier, all apparently healthy oaks with a five % or greater chance of being infected. A number of healthy oaks are thus sacrificed to ensure containment of the pathogen.

Oak wilt spread data, collected from pure stands of northern pin oak (*Q. ellipsoidalis* E. J. Hill) in Menominee County, Michigan, have been used to mathematically model the probability of oak wilt spread between diseased and healthy trees growing on Grayling or Pemene series soils (Bruhn et al. 1991). The resulting model, and the working tool derived from it, are described elsewhere in these proceedings by Bruhn and Pickens. Prior to model development, our desire to save trees led us to locate many barriers too close to diseased trees. These barriers averaged much less than 95% probability of disease containment. The result of such errors is "failure to contain" the pathogen.

Oak root systems in Michigan do not generally appear to grow beneath long-established, well-traveled roads. We have not seen root graft spread across even a hard packed gravel road. No additional barrier may be needed along a roadway.

Barriers are marked with flagging to guide the plow operator or fumigant applicator, and are emplaced in August following peak symptom expression. Barriers should be flagged ahead of time, because proper barrier location usually takes longer than barrier installation. Knowledge of the locations of buried utilities (e.g., telephone, electric, water and septic) is essential for proper barrier location and emplacement. Because of the meandering nature of

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barrier lines, someone familiar with the flagged lines should be on hand to guide the operator during barrier installation. Barriers should be created before trees in the focus are felled. Otherwise, water tension released when trees are felled may result in disease spread to grafted healthy trees beyond intended barriers.

Vibratory Plow

Root grafts are easily severed with a vibratory plow blade. Vibratory plow blades pulled by tractors are used to lay telephone cable. Such a unit may be hired at an hourly rate to install root graft barriers located by a professional forest pest management specialist familiar with oak wilt. Contracts for creating vibratory plow root graft barriers should be finalized in the winter or spring before placement. The contract should specify availability of the maximum number of hours needed and a time frame for completion, allowing down time for equipment repair, poor weather, and treatment of additional discovered foci. Development of contract language should be suggested by a pest management professional.

The contractor should be educated about the sensitivity of the environment within which he operates. This is especially important in residential and recreational areas, or areas where residual timber or landscape values are of concern. Wounding residual trees during barrier installation, especially those outside the inner barrier, must be avoided. Environmental impact also can be reduced by specifying that the tractor be outfitted with pads between the cleats on at least the left-side track, to avoid damage to roadbeds, and skid plates beside the blade at ground level, to minimize site disturbance.

The pathogen may avoid barrier containment if the barrier is too shallow to severe all roots, or if the barrier is established within the perimeter of infected roots. A plow blade capable of cutting a five-ft-deep barrier should be used. Four-ft blades are available, but will provide less satisfactory results. Units of local government undertaking an oak wilt control program should consider locating or purchasing a five-ft blade.

Large trees may be grafted to depths greater than five ft. To be most certain of separating the root systems of large trees, place the inner barrier just outside border trees left inside the inner barrier, rather than midway between trees. This practice will reduce the windfirmness of the trees just inside the inner barrier, and may result in blowdown in open areas. Therefore, if the locations of these trees result in structural hazard (e.g., proximity to dwellings, etc.), it is especially advisable to arrange for the removal of these trees soon after barrier emplacement.

Fumigants

In urban settings, with numerous underground utilities, a soil fumigant such as Vapam® should be used to chemically separate grafted root systems (Michigan State University Cooperative Extension Bulletin E-2022). If fumigant is used, it must be used strictly according to the directions on the product label. One drawback associated with use of soil fumigants is that some fumigants (e.g., Vapam®) can cause long, narrow cankers extending from below ground upward for several feet or more above ground on the barrier side of healthy oaks. The closer a tree is to the Vapam® barrier, the more likely it will become cankered. While these cankers typically do not kill trees, they are unsightly and reduce tree health.

Herbicides

Herbicides are useful in two ways for oak wilt control. First, if a new (single tree) oak wilt focus is diagnosed within a few days of upper canopy infection, and before the infection can reach the root system, frill girdling with herbicide treatment or felling may prevent the fungus from reaching the root system to spread (author's observation). Girdling should be done near the ground, and preferably with a chain saw to completely sever the outer rings of wood. The resulting wound is washed with an herbicide registered for cut-surface treatment of oak. Use only herbicides that are registered for this purpose, and carefully follow the label directions. Second, herbicides also may be used to preserve the integrity of root graft barriers. Trees within the inner barrier eventually become infected. If the disease takes several years to kill these trees, the fungus may cross the barrier through newly-formed root grafts.

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Removing all live trees from inside the inner barrier, followed by removal or herbicide treatment of stumps to prevent sprouting, prevents re-grafting of roots across the barrier. Infected trees must be carefully identified for sanitary treatment, and the barrier must be constructed before herbicide treatment or clearing are undertaken.

We have yet to find an herbicide which kills red oak root systems quickly enough to create reliable root graft barriers. Death of stems and canopies alone does not prevent oak wilt spread across the living root system of a poisoned tree and into the next healthy tree.

Preventing Overland Spread

The inefficiency with which insects vector the oak wilt fungus represents an exploitable weakness in the oak wilt disease cycle. The three key objectives of a program to prevent overland establishment of new oak wilt foci are; 1) to prevent wounding of healthy oaks during the spring and early summer, 2) to prevent people from moving diseased oak wood to locations near healthy oaks, and 3) to prevent mat formation, or at least to deny vectors access to mats.

Wounding Healthy Trees

Cutting restrictions during May and June are very helpful in the affected oak resource, until all diseased trees can be sanitarily treated or disposed. The exact period for cutting restrictions should cover the peak period of mat production. During this period, all activities which might wound healthy oaks should be postponed, including pruning, climbing oaks with climbing irons, posting gates or signs on living oaks, and tree removal or harvesting.

Use of Wound Paints

When feasible, wounds (natural and manmade) on healthy oaks should be treated immediately with a wound paint (Juzwik et al. 1985). While wound paints have been shown to have little or no value for preventing wood discoloration and decay (Shigo and Wilson 1977), insects are prevented from vectoring the oak wilt fungus to fresh wounds that are painted.

Transporting Wood from Diseased Trees

Oak firewood is commonly made from dead or dying trees, without concern for the cause of death. In areas where oak wilt occurs, oak firewood with intact bark should not be moved within the area or to other areas with valuable oak resources.

Sanitation

Oaks already dead for more than a year need not be treated, because they can no longer produce mats. After root graft barriers have been constructed, all oaks inside the inner (95%) barrier should be properly treated as quickly as possible to eliminate the chance of overland disease spread. Treatment must be completed before fungal mats can form, usually by the following April. There are several sanitary treatment options available for wood from wilting or recently-killed trees. First, diseased logs or firewood with attached bark can be thoroughly tarped on site, to the ground, with plastic sheeting. The tarp should be sealed around the base to prevent insects from reaching fungal mats. Thorough tarping deprives vectors of access to mats which may form, and competing fungi will kill the oak wilt fungus. Tarps may be removed at the end of the following summer.

Second, wood cut from infected trees can be moved to sites far removed from oaks (several miles should be sufficient), rendering vector transmission extremely unlikely. This option requires the careful supervision of oak wilt experts who are familiar with the local distribution of oak. Third, debarking or destruction (burning, chipping, milling, etc.) of infected wood before mats can form (by May 1) is an effective means of sanitation. The wood may be sold to a saw mill or chipping facility, preferably one which is several miles away from the nearest red oak. The purchaser must understand that the wood is diseased, and that it needs to be utilized over the coming winter. Ideally, all branches and wood over 3 inches in diameter should be treated. Mats do not form on lumber.

Apparently healthy red oaks within the inner barrier, which have not shown any disease symptoms by autumn of the year barriers are emplaced, should be felled or killed as soon as possible after barrier

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establishment. These trees can be marked exempt from sanitary handling, because mats do not form on trees which die before they are colonized by the oak wilt fungus. We recommend also that their stumps be killed with an herbicide, to protect barrier integrity by discouraging root growth within the barrier.

ACKNOWLEDGEMENTS

Adapted from Northern Journal of Applied Forestry 9:47-51, published by the Society of American Foresters, 5400 Grosvenor Lane, Bethesda, MD 20814-2198. Not for further reproduction.

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Cooperative Oak Wilt Program

OAK WILT SURVEY AND DIAGNOSIS FORM

I.D. Number _____

Observer_____ Agency_____ Date _____

Obs. Address _____ Phone _____

County _____ Political Township_____

T_____ R_____ Sec._____ Q' Sec. _____ Sample Sent? (Yes, No)

Forest Compartment Stand, If Applicable: _____, _____, _____.

Landowner's Name: _____

Address : _____

City,State,ZIP: _____

Phone Number: (___) _____

Background
(Circle or Complete Response)

1. Is this an area (within 10 mile radius) with confirmed oak wilt? (Yes, No, Unk.)
1a. If Yes, How Far?: _____ Miles.
2. Has this area suffered severe drought within the past 3 years? (Yes, No, Unk.)
3. Have the oaks in this area been severely defoliated in the past two years? (Yes, No, Unknown)
4. How many trees have died in this patch? (1, 2-10, 11-20, >21)
5. How many trees are currently wilting in this patch? (1, 2-10, 11-20, >21)
6. Did affected trees die more or less simultaneously (2 - 3 years)? (Yes, No, Unknown)
7. Are there any healthy twigs or branches present in otherwise dead portions of the crown? (Yes, No, Unknown)
8. Do recently killed trees retain most of their leaves? (Yes, No, Unknown)

Risk Data
(Circle one per group)

Land Use/Ownership
1. Primary Residential
2. Secondary Residential
3. Park or Campground
4. Private Non-industrial Woodlot
5. Public or Commercial Forest

Stand Type
1. Mixed oak stand with other tree species
2. Mixed red and white oak
3. Pure red oak stand

Evidence of Disease
1. Single dead tree - no pressure pads noted
2. Single wilting tree
3. Single dead tree - pressure pads found
4. Group of dead/dying trees - no pads noted
5. Group of dead/dying trees - pads found

Stand Size
1. Single tree or small group
2. Stand size less than .5 acre
3. Stand size .5 to 5 acres
4. Stand size greater than 5 acres

(OVER)

(Ver. 6/92)

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Diagnostic Key

(Circle your choice in each couplet visited.)

1 Tree is dead. (Go to couplet 2.)
1' Tree is wilting. (Go to couplet 9.)

2 Current year mortality. (Go to couplet 3.)
2' Previous year's mortality or older. (Go to couplet 6.)

3 Wood surface moist and light-colored; bark still well-attached. (Go to couplet 4.)
3' Wood surface moist and dark brown; bark loosely attached. (Go to couplet 5.)

4 Gray-blue streaking evident in the otherwise light-colored sapwood. **Submit sample of attached bark over streaked wood for culturing, and wait for results.**
4' No gray-blue streaking evident; wood is normal healthy light color. **Re-evaluate next May.**

5 Smell freshly exposed wood surface; no diagnostic fruity odor. **Re-evaluate next May.**
5' Smell freshly exposed wood surface; diagnostic fruity odor present. **Submit sample of attached bark over wood (taped together if necessary) for culturing, and wait for results.**

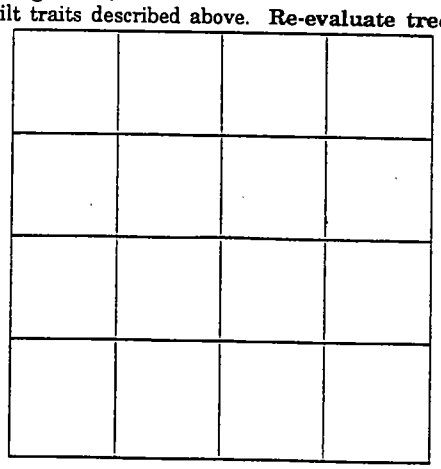
6 Wood surface moist and dark brown; bark loosely attached. (Go to couplet 7.)
6' Wood surface dark brown and drying; bark loose or sloughing. (Go to couplet 8.)

7 Pressure pads definitely found (search beneath cracks in bark). **OAK WILT.**
7' Pressure pads not found. **Submit sample.**

8 Black elliptical smears (old pressure pads) on wood surface under bark. **Probable oak wilt. Do not sample from this tree; reevaluate site in late July or August if new mortality develops.**
8' No remnants of old pressure pads found. **Do not sample from this tree; re-evaluate site in late July or August if new mortality appears.**

9 Wilt progresses downward, involving the entire crown; leaves quickly carpet the ground beneath affected trees in mid-summer; fallen leaves display "bronzing" from the tips back toward the base, with a distinguishable border between bronzed and green portions. **Probably oak wilt (go to 4).**
9' None of the oak wilt traits described above. **Re-evaluate tree in September.**

Vicinity Map: N↑



Scale: 3/4 inch = _____ feet, yards, chains, miles (circle one)
Confirmation Process (Circle One): Field Laboratory Non-confirmed
Field\Lab Confirmation By: _____
Date Confirmed: _____
Notes:

- I.D. Number Format: YYINI(Initials)0001 (Example: 91NWR0001)
- Submit Form and Samples ASAP to: Dr. P. Sood (517-337-5091), MDA Laboratory, 1615 S. Harrison Rd., East Lansing, MI 48823

Participating Agencies:
DNR MDA
MSU\CES MTU
USFS\SPF

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Oak Wilt Management in Minnesota

D. W. French

Although a serious threat to one of Minnesota's most valuable hardwood resources, the impact of oak wilt, caused by *Ceratocystis fagacearum*, is a problem that can be successfully managed when compared to other tree maladies. Various state agencies have joined efforts with tree care companies and community leaders to deal with oak wilt in Minnesota. The program has successfully promoted the cause of oak wilt control and played a key role in public education. This same forum has been used to promote issues of general tree health and fostered greater respect for the value of urban trees. Root disruption, firewood control, wound management, and reducing inoculum formation comprise the basis for disease control. There is promise for additional control measures, such as biological control and direct control with injection of chemicals, in the near future.

INTRODUCTION

Oak wilt in Minnesota and the surrounding states of Wisconsin, Michigan, Iowa, and other north central states has been a problem for many decades although not recognized until 1942. Carl Hartley, in his notes from 1912-1938, very accurately described the disease now known as oak wilt. "Large numbers of black oak *Quercus velutina* Lam. were dying in Albert Lea, Minnesota; also in an area 5 miles west of Minneapolis to 10 mi east of St. Paul, 10% of what were reported as black oaks were dead with more dying." Very likely these were northern red oaks *Q. borealis* Michx. and northern pin oaks, *Q. ellipsoidalis* E.J. Hill. It was noted that the dead trees were in groups which is characteristic of oak wilt.

A more recent survey, based on aerial photography, found over 3,000 oak wilt infection centers involving approximately 100,000 trees. Oak wilt has been found in 18 southeastern Minnesota counties. In addition, it has been found in a few trees in Aitkin, Crow Wing, Cass, and Stearns counties and possibly in others not yet confirmed. The fungus has been introduced to these outlying locations by people transporting firewood. That is how the fungus reached the upper peninsula of Michigan, having been brought there on firewood from Milwaukee, Wisconsin. Undoubtedly, there will be more introductions of oak wilt on firewood. Oak wilt is present in Wisconsin counties east of St. Paul and south to Illinois. It also has been found in northeastern Wisconsin.

Oak wilt was not recognized as such until 1942 by Birch Henry. It was assumed that oaks couldn't tolerate civilization. The name of the fungus that causes oak wilt was changed as more was learned. The current scientific name is *Ceratocystis fagacearum* (Bretz) Hunt. Previous names were *Chalara quercina* Henry and *Endoconidiophora fagacearum* Bretz.

Dutch elm disease, caused by *Ophiostoma ulmi* (Buism.) Nannf., was the first major tree disease for the urban forests of Minnesota. Although oak wilt was killing large numbers of oaks, the elms, which were the most numerous trees found in boulevards, parks, golf courses, and residences, were being annihilated by Dutch elm disease. Thus little attention was paid to our oaks which were in reality always more important than any of our other shade trees.

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Similar stories were occurring in all the north central states and major programs are now under way to save our oaks. The lessons learned from Dutch elm disease have helped everyone know more about how to develop programs to save and even increase our populations of oaks in urban settings. The organizations which evolved to help save our elms have now turned to the task of saving our oaks. The public has been educated or enlightened about trees, their value, and the importance of management programs to insure that oaks will be enjoyed by future generations. With the advent of ash yellows, caused by unnamed mycoplasmalike organisms (MLOs) which is now established in this part of the country, oaks are even more important in our urban forests.

STRATEGIES TO SAVE OUR OAKS

The public is receiving information about oak wilt from newspaper stories, extension publications, radio programs, TV shows, and presentations to county, state, and other audiences about how to save our oaks. Some counties have added personnel to their staff to work with communities in developing effective control programs.

Probably even more important has been the formation of the Minnesota Shade Tree Advisory Committee whose membership includes city foresters, tree care companies, personnel from the State Department of Agriculture and the State Department of Natural Resources, representatives from the University of Minnesota, and county representation including extension staff. This group was formed in the early 1970s in response to Dutch elm disease and to promote more effective tree programs for the state.

The Committee continues to grow and attendance has remained high. The regular meetings keep everyone up-to-date with the newest information and provide an opportunity for each group to inform the entire task force about what is happening all over the state on urban tree issues. The meetings have been well attended and, as a result, all the communities, from large cities to small towns, are well informed about new programs, opportunities for funding, and how best to solve the tree problems they are facing in their community. This Committee has been most helpful in encouraging the legislature to fund urban tree programs.

This year a program was arranged to show key legislators and members of Congress the impact that oak wilt is having on many communities. These communities rely primarily on oaks to provide shade and beauty for their parks and for their urban forests. Those attending were impressed with the impact oak wilt was having on towns, new housing developments, parks, and the overall forested areas.

An excursion and deomstration on July 8, 1991, included city mayors, township board members, city council members, county commissioners, and state legislators. Forty-three key legislators on the LCMR Committee attended a summary of what we are trying to do to protect oak forests and they saw a demonstration of the vibratory plow. The bus tour covered residential areas devastated by oak wilt. Everyone was impressed with the impact being made by oak wilt. All five major networks covered these programs. The vibratory plow is an impressive machine and effective in stopping the fungus from spreading from tree to tree via root grafts.

We have learned that road builders are very interested in learning how to reduce the losses to oaks and avoid spreading the disease, and how, in fact, they can reduce the amount of oak wilt without much added effort on their part. By following our recommendations, they find that few trees are lost and people in the community are pleased with the results. The Anoka County Highway Department had 27 people attend the education program and 340 realtors earned credit hours for attending.

Each year we have talked to builders' associations -- in fact, they pay for the program. They are impressed that tree losses can be drastically reduced with no added expense to them. Just knowing the facts about oak wilt makes this task much easier. Not only are we able to eliminate law suits (where people contest their property has been damaged) but more important, people are pleased with how the trees were protected at little additional cost. As a result of this program, home builders and general contractors are more apt to request advice before the project is started; thus, the right decisions are made about which trees to save and which to remove. Obviously, the people acquire a great deal

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of useful information at no cost to them but as a service provided by the contractors.

EDUCATION

At least once a year we have programs to teach those involved in any aspect of the tree business how to deal with oak wilt, Dutch elm disease, ash yellows, storm damage, etc. These programs also provide education on insect pests, diseases, and all other aspects of tree care. These people are required to pass a test and in subsequent years are required to attend updating sessions so they have the most recent recommendations for whatever phase of tree care in which they may be involved. This includes tree service companies and nurseries. The more people know about oak wilt and all the other problems of trees, the better our landscapes and urban forests will serve all of us.

We have had several contacts with pipeline companies who do business in Minnesota, especially regarding their construction of pipelines. On one occasion we lectured to their employees meeting in Texas. The program included a question and answer session where we could tell them how to avoid oak wilt problems. On another occasion, some of their people attended a lecture on oak wilt with our forestry students. We are in continuing contact with all companies involved in furnishing electric power and they are following our recommendations.

OAK WILT SURVEYS

The original surveys for oak wilt were done as part of other projects and thus we were aware of the approximate range of the disease. In July 1988, the Minnesota Department of Natural Resources in conjunction with the University of Minnesota photographed 1620 square miles which involved 44 townships all located north of the Twin Cities of Minneapolis and St. Paul (Oak Wilt in Minnesota, by D.W. French and T.G. Eiber published by the Minnesota Department of Natural Resources - 28 pages). This was the area in Minnesota where most of the oak wilt occurred. The survey for the balance of the state has been accomplished by direct aerial mapping and by car surveys. Over many years the University has received hundreds of samples which were tested for the fungus.

Oak wilt has been found in counties north of where major centers of infection occur and in each case we have reasonable evidence that the fungus was brought to these locations by people transporting firewood or simply getting rid of oak brush. By way of publicity we are attempting to stop the movement of oak material unless it is cleared by a state agency. Logs or firewood from recently wilting oaks in spring (primarily between May 15 and June 15) can have mats which, if inhabited by the vector Nitidulidae (picnic beetles), can be the source of inoculum for healthy oaks. The healthy oaks must be recently wounded (May 15 - June 15) for them to be inoculated by these beetles. We are fortunate in that Nitidulidae do not prefer oaks and are attracted to a wide range of habitats other than oaks.

THE FUTURE OF OAK WILT IN THIS PART OF THE U.S.

We are confident that oak wilt is a disease which can be greatly reduced. The future of the oaks, in spite of oak wilt, is a great deal brighter than the future of elms. We have control strategies which are effective, financially within reason, and we are certain we can gain the upper hand and gradually reduce oak wilt to a disease with minor impact on our urban forests.

Because oaks are often grafted to adjacent oaks via root grafts, this means of spread from tree to tree must be stopped. Fortunately, this can be accomplished at a reasonable cost. Vibratory plows can be contracted for root disruption and infection centers can be isolated from the surrounding healthy trees. Once this is accomplished, the fungus can spread only by certain insects, primarily the Nitidulidae, which are not necessarily attracted to oak trees and may prefer other habitats. These insects can not wound trees and thus wounds must be made by another agent, usually people pruning trees. These wounds are susceptible from about May 15 to June 15 and if older than six to eight dys., are not susceptible. If wounds are painted with a non-toxic paint, infection will not occur. Simply by not wounding oak trees (between May 15 - June 15), new infections can be prevented. These dates may vary in other states.

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We have demonstrated that if oak wilt mats are sprayed with spores of the fungus *Ceratocystis piceae* (Munch) Bakshi, the oak wilt spores are not able to cause infection. Undoubtedly, other chemicals or fungi might prevent sporulation. Oak trees that are potential sources of mats in the spring can be felled and wrapped in plastic. These logs in plastic will be overrun by *Trichoderma* spp. and other fungi which prevent the oak wilt fungus from producing viable spores. After the critical period in spring, these logs can be unwrapped and used for firewood without endangering healthy trees.

The oak wilt fungus can be used as a selective silvicide to eradicate undesirable oaks. In wildlife habitats, oaks often serve as perches for predators and have contributed to the loss of much wildlife. In comparison with chemical silvicides and fire, the oak wilt fungus is easier to use and much more effective. The fungus has been used as a selective silvicide in Arkansas.

By using sodium arsenite on a girdle near the base of the main stem, spring mat formation is eliminated or the average number of mats per tree will be much fewer. The duration of new mat production was much shorter and mat size was smaller than on nontreated trees. Dry girdling of oaks which wilted in July prevented mats from forming on these trees the following spring. Mats did form on some oaks that were dry girdled in August. This technique was not used, simply because girdling these trees increased the possibility of the trees falling and causing damage to buildings and people.

Recently a treatment involving a compound called propiconazole manufactured by Ciba-Geigy has been effective in preventing infection of healthy oaks surrounding the infected oak, and in the recovery of the trees already infected with the fungus. More studies are needed to fully evaluate the effectiveness of this product.

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Oak Wilt Management in West Virginia

S. C. Haynes

A survey and control program for oak wilt (*Ceratocystis fagacearum*) was conducted from 1951 through 1977 in West Virginia. During this 27-year period, 57,740 actively wilting trees were detected for an average of 2,139 trees per year. The deep girdle technique was employed to treat diseased trees in order to reduce disease spread. Based on the results of three studies, beginning in 1958 and continuing through 1973, this procedure appeared to provide only marginal, if any, control. A cooperative pilot project with the USDA Forest Service was initiated in 1974 and continued through 1976 to test the feasibility and effectiveness of using cacodylic acid (dimethylarsenic acid) as an oak wilt control agent. The technique showed promise, but was time consuming and labor intensive. Oak wilt is still considered a potentially devastating disease in West Virginia. There are plans to continue to monitor disease incidence.

EARLY HISTORY

The discovery of oak wilt, caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt, in Pennsylvania and other states outside the Upper Mississippi Valley region in 1950 aroused concern among foresters, pathologists, and conservationists in West Virginia. At that time, over 70% of the land area in West Virginia was forested. This amounted to almost 9.86 million ac of commercial forest land. Oak comprised 60% of the forested area (Wray 1952). The major oak timber species in West Virginia included northern red (*Quercus rubra* Michx.), black (*Q. velutina* Lam.), scarlet (*Q. coccinea* Muenchh.), white (*Q. alba* L.), and chestnut oak (*Q. montana* Willd.). Since oak wilt followed chestnut blight, there was a great deal of concern that a fate similar to what had happened to the American chestnut (*Castanea dentata* (Marsh.) Borkh) would befall the oaks.

Concern for the health of the oak resource resulted in a meeting of representatives from the West Virginia Department of Agriculture (WVDA), West Virginia Conservation Commission, West Virginia University (WVU), United States Department of Agriculture, and industry in May 1951 to plan an oak wilt survey for West Virginia. As a result of this meeting, the West Virginia Conservation Commission organized crews of ground scouts to survey on foot and by car. Additionally, a Conservation Commission airplane was utilized to conduct aerial surveys. In 1951, 54 diseased oak trees were detected. No control program was yet in place, so the crews were only involved in survey and detection (True et al. 1951). Not only did the 1951 survey document the fact that the oak wilt pathogen occurred in West Virginia, but it also demonstrated a need for field and laboratory research directed toward an efficient and effective control (True et al. 1951). Therefore, a West Virginia Oak Wilt Committee was formed to raise funds to support and expand a research program at WVU. The committee was composed of timberland owners and individuals from the wood-using industry.

The results from the 1952 and 1953 surveys were similar to those of the 1951 season. Fifty-seven

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actively-wilting trees were found in 1952 and 114 were found in 1953. Each of these years, approximately 10% of the state was surveyed. In addition to the survey activities in 1952, a control program was initiated. The method employed was to cut and burn all infected trees detected by the survey and to poison the root systems of all healthy oaks within a 50-ft radius (Gillespie and Craig 1957). The herbicides employed were ammate (ammonium sulfamate) and 2,4,5-T (2,4,5 trichlorophenoxyacetic acid).

During this time, researchers at WVU were investigating a control technique known as deep-girdling (True et al. 1955). The technique involved girdling the tree to the heartwood with an axe at a convenient height above the ground. The bark was then peeled off the tree from the girdle to the ground. This technique was shown experimentally to cause the tree to dry out, greatly reducing the chance of fungal mat formation. Researchers assumed that if fewer fungal mats were produced, there would be a corresponding reduction in long-distance spread.

The deep-girdle technique was used experimentally on a number of the diseased trees detected in 1954. Additionally, there was experimentation involving the injection of copper sulfate into diseased trees. Both techniques appeared to reduce fungal mat formation, but the deep-girdle technique gave slightly better results. The remaining diseased trees were cut and burned (True et al. 1955). At this time, researchers discounted the role of local or root graft spread in West Virginia (Staley and True 1952). Later studies suggested that only 10% of oaks in the red oak group were root grafted to nearby susceptible trees (True et al. 1960).

By 1955, the West Virginia legislature was convinced of the urgency of the oak wilt disease problem and appropriated the first state funds for the survey and control program. However, it was still impossible to cover the entire state. The deep-girdle technique was adopted as the control method employed by the West Virginia Oak Wilt Program. With funding, there was an increase in the amount of aerial surveillance conducted and the number of ground scouts. With a more intensive survey, a greater number of actively-wilting trees was detected. In 1955, 795 actively-wilting trees were detected.

The Federal government was petitioned in 1957 for funding to assist with the survey and suppression of oak wilt. Under the Forest Pest Control Act of 1947, the Federal government could cost share up to 33.3% on programs to control forest disease problems. The Federal government agreed and supplied funding at the 33.3% rate.

EFFORTS TO EVALUATE CONTROL TECHNIQUES

Unfortunately, no good method existed to evaluate the effectiveness of oak wilt control methods employed by various states. Therefore, in 1958, the USDA Forest Service (USFS) developed a five-year study that involved the establishment of plots to evaluate different treatments. These plots, called Post Control Appraisal (PCA) Plots, were established in Kentucky, Maryland, North Carolina, Pennsylvania, Tennessee, and West Virginia. Each plot was circular, 2.9 ac in size with a diameter of 400 ft. An oak wilt infection center was located as close as possible to plot center and each center could not consist of more than five wilting or wilt-killed trees. At least five healthy oaks of the red oak group, two in d.b.h. or larger, needed to be within 50 ft of the infected oaks and each infection center had to be at least 500 ft from any other infection centers (Jones 1965).

Different control techniques were employed by each state or groups of states. Kentucky felled diseased trees, sprayed them with the persistent insecticides DDT (dichloro diphenyl trichloroethane) and BHC (benzene hexachloride) in fuel oil and treated stumps with the herbicide 2,4,5-T. North Carolina and Tennessee used identical survey and control methods, and therefore were grouped as one unit. They used the same insecticides as Kentucky, but added the fungicide pentachlorophenol. Additionally, the herbicide ammate was applied to cut stumps.

In Pennsylvania, diseased oaks and oaks of the same species group as the diseased tree within 50 ft of the diseased tree were felled. All stumps were treated with ammate. The deep-girdle treatment was used in West Virginia plots. The check plots for both the Pennsylvania and West Virginia programs were placed in western Maryland. It was felt that it was

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too risky to leave untreated plots in high disease incidence areas like Pennsylvania and West Virginia.

Analysis of the results from the data collected between 1958 and 1963 suggested that oak wilt spread was reduced by control programs in Pennsylvania (76%), West Virginia (50%), and North Carolina/Tennessee (42%). No control was achieved in Kentucky. In addition, the rate of fungus spread on check plots where no control activities were undertaken increased with time. However, due to the erratic nature of the disease, it was impossible to predict with great confidence what future disease losses might be for any area not under control (Jones 1965).

A second PCA study, four years in length, was initiated in 1964 to correct some of the design flaws in the first study. In the second PCA study, only the Pennsylvania and West Virginia treatments were compared. Additionally, the West Virginia and Pennsylvania treatments were used in both states. Check plots also were established in both states. Plots from the initial PCA study were maintained as an adjunct to the main study. The results from the second PCA study didn't agree with those of the first. The second study demonstrated that there was a tendency for oak wilt centers to become less active over time.

The Pennsylvania method was extremely effective at controlling oak wilt spread, but at a high price in healthy trees destroyed. The West Virginia deep-girdle method was less effective in either state compared to the Pennsylvania method. Still, in southern West Virginia, disease spread was reduced by 79%. However, only marginal control (39%) with this technique was reported in Pennsylvania and no effect was reported in northeast West Virginia (Jones 1971).

A new study entitled the Modified Control Appraisal (MCA) study was initiated in 1969. It was designed to measure the amount and importance of long-distance spread of the oak wilt fungus and determine how effective the West Virginia method was in reducing this type of spread. Ten study areas, each approximately 232 square mi in size, were established, five in southern and five northeastern West Virginia. These study areas were quadrangles, 15 min in latitude by 15 min in longitude. Four quadrangles, two each in southern and northeastern West Virginia, received no treatment while four other quadrangles, two each in southern and northeastern West Virginia, were treated using the deep-girdle technique.

The two remaining quadrangles were used for felling diseased trees to investigate insect activity. The MCA Study was completed in 1973. The results did not differ significantly from those of the second PCA study. Girdled and non-girdled quads had similar fluctuations in numbers of diseased trees found. From both the PCA and MCA studies, there was a growing realization that root graft disease transmission was a more important component to disease spread in West Virginia than originally believed. Historically, approximately 50% of the wilting trees occurred within 50 ft of previously diseased trees (Gillespie and True 1959).

Testing was conducted in 1973 and 1974 using a new technique to control root grafts. Wilting red oaks in northeastern West Virginia were injected with the herbicide cacodylic acid (dimethylarsinic acid), an organo-arsenical compound. This work demonstrated that 31% of the diseased trees were root grafted to healthy trees. Root grafts also were observed between red and white oaks. Additionally, root graft transmission might take several years to occur (Rexrode and Frame 1977).

Since the new root graft control technique showed promise, a cooperative pilot project with the USFS was initiated in 1974 and continued through 1976 to test the feasibility and effectiveness of using cacodylic acid as a chemical to control oak wilt by minimizing root grafts and insect spread. Cacodylic acid acts as an insecticide as well as an herbicide. It was pressure injected into diseased trees. This technique was employed in northeastern West Virginia on the same 15-minute quadrangles from the MCA study where deep girdling previously had been used. Two quadrangles were used as checks.

The procedure showed some promise. There was a net reduction of 48% in diseased trees and a net reduction of 38% in numbers of active centers over the last two years of the study (Rexrode 1977). The injection technique, however, proved to be labor intensive and time consuming. A crew employing this technique could treat only half as many trees in a given time period as a crew using the deep-girdle procedure. It also required a great deal of effort to

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carry the injection equipment, jugs of water, and cacodylic acid into West Virginia's rugged, mountainous terrain.

During the mid-1970s, the WVDA also cooperated with researchers at WVU in evaluating other chemicals and biocontrol agents for oak wilt control. Chemical compounds tested included potassium iodide, copper sulfate, and combinations of copper sulfate/cacodylic acid solutions. Biocontrol agents evaluated included three fungi: *Schizophyllum commune*, *Trichoderma viride*, and *Gliocladium roseum*. Each of the treatments effectively reduced the colonization of the host by the oak wilt fungus and subsequent fungal mat production on the host. However, the same costly time restrictions were involved as occurred with the cacodylic acid injection procedure. None of the chemical compounds or biocontrol agents was evaluated for its long-term effect on disease incidence.

At the end of the 1976 summer season, all federal funding for the West Virginia oak wilt project was discontinued. In light of the results from the second PCA study and the MCA study, this program was no longer considered cost effective. The WVDA continued the survey and control program through the 1977 summer season and then terminated the program. Lack of cost effectiveness was given as the reason.

PERSPECTIVES AND POST CONTROL PROGRAM ACTIVITIES

During the 27 years when the annual oak wilt survey and control program was conducted in West Virginia, 57,740 actively-wilting trees were detected with an average of 2,139 trees per year. The fewest number of actively-wilting trees were detected in 1951 when 54 trees were located. The greatest number of diseased trees were found in 1963, when 3,937 actively- wilting trees were located. The most intensive surveys were conducted in the 1960s when nearly 100% of the state was surveyed annually. During this period, an average of 3,150 trees were detected each year. Oak wilt was never detected in Brooke, Ohio, Tucker, or Webster counties by the WVDA survey and control program.

Several problems were encountered when the program was active. Inclement weather affected aerial observer visibility, reduced flying time, and slowed ground scouting activity. Emergences of the periodical cicada made field diagnosis of symptomatic trees extremely difficult. Likewise, on other occasions, late spring frosts in June hampered symptom diagnosis.

During the entire program, there always was a problem finding adequate personnel to work as ground scouts and aerial observers. Additionally, it was difficult to find skilled pilots to fly at the low elevations in rugged terrain required to spot individual infected trees. A total of nine airplane accidents occurred during the 27-year history of the program. Three pilots and two aerial observers died as a result of three of the accidents.

From 1979-1985, aerial surveillance was maintained over four of the northeastern 15-minute quadrangles used in the MCA and injection projects. The number of diseased trees detected varied greatly from year to year. Historically, there always was a great deal of fluctuation in numbers of diseased trees sighted on most quadrangles. No apparent trends for increases or decreases in disease incidence were evident. Due to continuing state financial problems and rising costs, the aerial oak wilt survey was discontinued in 1985.

In 1982, a study was conducted to evaluate the incidence of local spread of oak wilt during 1970-1982 in untreated centers and in centers treated by cacodylic acid injections and deep-girdle control methods. Forty-one oak wilt centers originally found in 1970-1973 in northeastern West Virginia were revisited to determine if there had been any dramatic changes in oak wilt incidence. At each site, the stump or stem of the originally-infected tree was located and a plot with a radius of 50 ft established around it. Data taken on each plot included basal area by species, number of oaks killed since the original discovery and number of actively-wilting trees. Neither cacodylic acid injections nor deep-girdling treatments affected the subsequent incidence of mortality compared with no treatment. Additionally, the total basal area per ac was affected little by oak wilt (Mielke et al. 1983).

In 1992, the forested area in West Virginia contained an estimated 12.1 million ac of timberland. Oak/hickory is the dominate timber type, occupying 77% of the timberland. Approximately 56% of the

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timber is oak (DiGiovanni 1990). Therefore, concern about the potential for oak wilt disease still exists, even though there has been no apparent increase in its incidence since the oak wilt control program was discontinued. This disease certainly has the potential to kill red oaks rapidly, but appears to lack an efficient vector. A change in the vector relationship could cause a dramatic change in disease incidence (Gibbs and French 1980).

The WVDA still views this disease as potentially very devastating and thus plans to periodically monitor disease incidence on some of the same quadrangles used in the MCA and pilot project studies. Additionally, there are plans to aerially survey two (Tucker and Webster) of the four counties where oak wilt has never been detected to determine if they are still oak wilt free. Limited ground scouting will be done on the quadrangles in northeastern West Virginia. Any potential disease centers in Tucker and Webster Counties will be confirmed by visits to the site.

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Oak Wilt Management in Pennsylvania

William Merrill

From 1951 through 1971, Pennsylvania conducted an intensive research program and state-wide survey-detection and "eradication" programs for oak wilt caused by the fungus *Ceratocystis fagacearum*. The cumulative number of oak wilt infection centers increased in a sigmoidal fashion and appeared totally unrelated to control efforts.

INTRODUCTION

Oak wilt was first discovered in Pennsylvania near McVeytown, Mifflin County, in 1950 (Fergus and Morris 1950). The number of dead trees at the locus suggested the disease had been present for some time. All dead and dying trees were immediately felled. Other infection centers were soon discovered throughout the south-central and southwest portions of the state, and with the general hysteria that prevailed at the time (i.e., another "chestnut blight epidemic" that was going to eliminate all oaks from North America), state-wide survey-detection and "eradication" programs were initiated. These programs were carried out by the Pennsylvania Department of Agriculture, Bureau of Plant Industry (PaBPI).

Simultaneously, three different groups initiated research programs: the PaBPI, the Pennsylvania Bureau of Forestry (PaBF), and Pennsylvania State University's (PSU) Department of Plant Pathology. PSU Department of Entomology was never involved; the entomological studies were conducted primarily by members of the PaBF under the direction of Dr. F. C. Craighead, and by members of the PaBPI. Initially, there was cooperation among all three groups, and PSU worked with both state agencies. However, as time went on increasing antagonism developed between PaBF and PaBPI due in part to infighting over "turf;" the PaBF deeply resented the fact that survey-detection and control on state forest lands were being done by the PaBPI.

Further, there were fundamental and unresolved differences in opinion as to the nature of oak wilt. Matters came to a head and policy edicts came down from the state administration. Both the PaBPI and the PaBF were told they had no research mandate; the only organization within the state with a research mandate was PSU. PaBPI held the mandate, under state law, to conduct survey and control programs for all dangerous pests on all lands within the state. PaBF was removed from involvement with oak wilt, although it was directed to provide PSU personnel with suitable research areas on state forest lands.

OAK WILT RESEARCH

Oak wilt research by the PaBF under the direction of Dr. Craighead involved entomologists B. H. Hadley, Jr., J. C. Nelson, and pathologist C. L. Morris. The PaBPI team consisted of entomologists A. R. Jeffery, H. E. Thompson, and pathologist W. L. Yount. Studies of both groups were directed primarily towards insect vectors, sources of inoculum, and infection courts.

Oak wilt research at PSU was initiated in 1951 by Dr. C. L. Fergus. Several students completed theses on oak wilt under his direction (Merek 1953, Cole 1955, Yelenosky 1958, Shain 1960, Bell 1964). W. A. Stambaugh, working as an instructor at PSU while he completed his Ph.D. *in absentia* from Yale

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University, also completed his dissertation on oak wilt (Stambaugh 1957). In 1963, Dr. F. A. Wood replaced Dr. Fergus. Lacasse (1966a) and Skelly (1968) completed theses on oak wilt under Dr. Wood. In 1965, Dr. W. Merrill replaced Dr. Wood. Skelly carried out several oak wilt studies with Merrill. Wertz (1970), and Peplinski (1972) completed theses on oak wilt under Merrill. Wilhour (1968) and Popp (1968) completed theses on oak wilt under Dr. R. Hutnik in the PSU School of Forest Resources. In addition, Bowen (Bowen and Merrill 1982) completed a "senior thesis" under Merrill. Although spread continues to be monitored in one infection center initiated in 1966 in Perry County (Merrill 1991), oak wilt field research terminated in December 1971.

THE PENNSYLVANIA OAK WILT CONTROL PROGRAM

The state-wide control program that evolved after 1951 utilized aerial surveys to locate infection centers. The crew usually consisted of a pilot and an observer who flew over the western half of the state and concentrated on the "oak wilt area", from June to early August. Areas of greatest oak wilt incidence were surveyed as many as five times per season. The observer marked the location of suspected centers onto a topographic map. Then a two-man scouting crew would locate and verify the center. If they ascertained that it was indeed an oak wilt infection center, and not a result of lightning or other causes, they then paint-blazed a straight trail out to the nearest road. At the roadside, a tree would be prominently paint-blazed, and also marked with an "OW" and the year of detection. Finally, the felling crew would follow the blazed trail to the center, and with axes and saws fell all dead and infected oaks, and all oaks of the same subgenus within 50 ft of dead or dying oaks.

This procedure was based on early studies in Iowa that indicated that local or short-range spread (that is, increase in size of existing foci) could be prevented by felling dead and dying trees, and healthy trees of the same subgenus within 50 ft (Dietz and Young 1948, Young 1949). Because about 97% of all infected oaks in Pennsylvania were of the red oak group (subgenus Erythrobalanus), this meant cutting a minimum of a 100-ft diameter group of red (*Quercus rubra* L.) and/or black (*Q. velutina* Lam.) oaks. The stumps of all felled trees were treated with herbicides to prevent sprouting and kill the stumps and their root systems. This would prevent root-graft transmission of the pathogen. In the early years of the program, sodium arsenite was used; because of crew safety, in later years Ammate[R] was used.

In the early 1960s, limited experiments were carried out involving the aerial bombing of infection centers with sodium arsenite. This resulted in killing all vegetation in a fair-sized area, and totally eradicated all infection centers thus treated. However, many of these sites had not revegetated by 1969-70.

An 8 x 10-in. record card was kept for every infection center in the state. These were keyed by a grid system that easily converted to latitude and longitude. Each card also contained the following information: county, township, detection date, verification date, scouting crew and leader, date treated, sketch map of the site, number of dead and wilting trees of each species of oak affected in the center, number of trees felled, degree of slope, aspect, etc.

The control program was under the direction of Mr. A. R. Jeffery for its duration. Scouting and felling crews were stationed out of Mount Union, Bedford, and McConnellsburg, Pennsylvania in the early years, and primarily out of Bedford in the final years of the program, as this was central to the majority of the infection centers.

Control was carried out on state and private lands. Scouting and felling crews carried I. D. cards allowing them access to all properties in the state, even those posted against trespass, except military bases and prison grounds. In a few instances, scouting and felling crews had to be escorted by armed state police while armed land owners watched. Land owners were allowed to carry out control on their own properties if they intended to harvest and utilize the trees.

The "oak wilt hot spot" in Pennsylvania lay in the south central region, primarily Bedford, Fulton, and Huntingdon Counties, and the western halves of Juniata, Mifflin, and Perry Counties. The disease did not occur east of Blue Mountain, a mountain ridge system arcing from the Susquehanna River just north of Harrisburg, Pennsylvania southwest to Hancock,

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MD. In 1965 the most northeastern infection center in the United States occurred along Pennsylvania Route 45 on the Centre-Huntingdon County line atop Tussey Mt. The disease has never been found further northeast, nor east of the Susquehanna River, and only uncommonly north of the Juniata River. The disease occurred sporadically throughout the southwestern counties of the state, primarily south of US Route 22. In recent years, the only known occurrences of the disease in Pennsylvania have been in the vicinity of Pittsburgh in residential trees pruned or wounded by climbers wearing spikes in late May or early June. All data regarding oak wilt incidence in Pennsylvania from 1950 to 1968 are summarized (Jeffery and Tressler 1969).

COMMENTS ON CRAIGHEAD & NELSON, 1960, OAK WILT IN PENNSYLVANIA

A "swan-song" paper by PaBF personnel (Craighead and Nelson 1960) was highly critical of the PaBPI survey-detection and control programs. It made several claims unsubstantiated by any published data, and severely criticized virtually all oak wilt research not done by the PaBF, especially entomological studies. This paper has never been rebutted and, as a still-cited summary paper, presents a somewhat distorted view of oak wilt in Pennsylvania.

Craighead and Nelson stated that the oak wilt pathogen was widely distributed in chestnut (*Q. prinus* L.) and white (*Q. alba* L.) oak. They further stated that this was not recognized by the survey and control teams, and that they believed these species were the primary source of inoculum. This was unsubstantiated by facts. In seven years of extensive field work in Perry County we found only two non-inoculated chestnut oaks with oak wilt.

Although a large proportion of the chestnut oaks had various degrees of branch and crown dieback, frequently with xylem discoloration, in no instance were we able to isolate the pathogen from the wilting branches, boles, or buttress roots of dozens of such trees. In all instances, the crown deterioration was associated with high populations of the golden pit-making oak scale, *Asterolecanium* spp. (Homoptera: Asterolecaniidae). High populations of these insects frequently were associated with xylem discoloration.

Furthermore, we inoculated 100 chestnut oaks spaced along three miles of the north slope of Bowers Mt. in Perry County. Each oak was inoculated in early June with 5 ml of conidial suspension containing approximately 4 X 10^4 spores/ml. Each was within 15 ft of a red oak with no intervening trees or shrubs, and in many instances the red oaks were growing between the flaring buttress roots of the chestnut oak. Approximately one third of the chestnut oaks died; the others suffered various degrees of crown mortality, then recovered. In the following five yr no red oak in the area wilted. In seven yr of study we were never able to document spread from chestnut oak to red oaks, and we never encountered infected white oak.

In studies to investigate the potential of American chestnut [*Castanea dentata* (Marsh.) Borkh.] to serve as a source of inoculum, we also inoculated numerous stump sprouts from two-to six-in. dbh over a three-yr period, with similar concentrated inoculum of *C. fagacearum*. No sprout ever developed any symptoms of oak wilt. All inoculated chestnut sprouts eventually died due to development of blight cankers initiated at the inoculation sites.

Craighead and Nelson stated that the oak wilt pathogen sporulated in wounds that could hold water on the sides of diseased red oaks and chestnut oaks, including infected but symptomless trees. The late Dr. C. L. Fergus stated that Dr. Craighead and his assisting entomologists pipetted water into and out of bole wounds with pipettes that he (Fergus) had used earlier that morning to cross conidiate some oak wilt cultures. Subsequent studies in Pennsylvania (Cobb et al. 1965) showed that the pathogen could sporulate in fresh wounds on red oaks at or near the mat-producing stage; 19% of the wounds on red oaks at or near mat-production stage yielded spores versus 2% of red oaks at other stages of wilt, significantly different at $P < 0.01$. Wounds three dy old yielded the pathogen more frequently than wounds one or seven dys. old.

Wound sporulation on chestnut oak was rare. In no instance was the fungus recovered from water trapped in the ends of hollow branch stubs, or similarly-aged wounds. These studies were

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confirmed in part in West Virginia (Amos 1965). The chances of getting fresh wounds of the type that could hold water on healthy oaks in late May-early June seems remote. It seems more likely that such wounds could be made by woodboring insects in weakened or dying trees.

However, Craighead and Nelson's criticisms of the costs of control *vis-a-vis* actual timber losses due to oak wilt were valid, as were some of their complaints regarding trees missed by the surveys. Trees infected late in the season that failed to leaf out the following spring or that shed their leaves early were missed by the survey. The average number of dead and dying trees in each Pennsylvania infection center over the year (about three dead trees and two dying trees) substantiates this fact. Their vehement criticisms of the techniques used in insect transmission studies also were valid. This point is discussed in this publication under "Insects and the Epidemiology of Oak Wilt."

STATE-FEDERAL POST-CONTROL APPRAISAL STUDIES

Two joint state-USDA Forest Service studies were conducted to evaluate the efficacy of the oak wilt control programs in Pennsylvania and West Virginia (Jones 1965). Neither state would allow the other's control program to be tested within its borders, and neither wanted infection centers to go untreated as checks. The West Virginia survey-detection program was similar to that of Pennsylvania, but the control consisted of deep-girdling dying trees, and debarking them from the girdle to the soil line. Therefore, in the first five-yr study the West Virginia control program was used in West Virginia, the Pennsylvania control program in Pennsylvania, and most untreated check plots were established in Maryland.

Later, some check plots were established in both Pennsylvania and West Virginia. Because of this flawed experimental design, there was no valid way to compare the individual control programs. The study suggested that oak wilt incidence tended to increase in untreated check plots, and that the Pennsylvania control program reduced local spread by 76%, whereas the West Virginia control program reduced local spread by 50%. But, as there were no valid controls upon which to base these analyses, these conclusions really were only conjecture.

In the second post-control appraisal study, Pennsylvania and West Virginia finally agreed to test each other's control program. Thus, in each state approximately one third of the study plots received no control, the Pennsylvania control, or the West Virginia control. The results of this study showed that oak wilt incidence declined in untreated infection centers. The Pennsylvania control almost totally eliminated local spread, and in nearly all cases where local spread did occur, it was due to faulty application of the control measures. However, on those plots approximately seven healthy trees were killed for every dead or wilting tree treated. The West Virginia control was less expensive to carry out, did not destroy healthy trees, and appeared to work fairly well in southern West Virginia, but had no effect at all in northeastern West Virginia and little effect in south-central Pennsylvania (Jones 1971).

Neither post-control appraisal study addressed the far more important question: did the control programs have any effect on the rate of establishment of new oak wilt infection centers?

SOME OTHER PERTINENT "SPREAD" STUDIES

The nonpersistence of infection centers should have been of no surprise. As early as 1955, studies in the Lake States, where root-grafting of oaks is far more common than in Pennsylvania and West Virginia, indicated that sanitation and isolation provided inadequate control (Anonymous 1956). Further, extensive studies in Illinois, also an area of more frequent root grafting than Pennsylvania and West Virginia, showed that one third of all infection centers were active only one yr, and another third died out within five yr (Himelick and Fox 1961). In Pennsylvania less than 3% of the red oaks have functional root grafts, primarily due to the mixed species composition of the forest.

From 1965 through 1968 in Perry County, Pennsylvania, only three of 1200 single-tree inoculations (red oaks) resulted in spread to other trees; of these, two centers were active for two yr, and the third was active for three yr. A single multiple-tree root inoculation study (red oaks) has

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continued to spread in Perry County for 25 yr. In the latter infection center trees appear to die at random, with no relation to previously-dead trees. All infection has occurred between the 1420 and 1460 ft contours on the east, northeast and north sides of a low hill. The pathogen has not affected numerous red oaks above or below these contour lines, and many healthy red oaks still remain within the plot, some within a few feet of red oaks that died.

PSU ANALYSES OF OAK WILT "SPREAD"

In Pennsylvania from 1953 to 1968, oak wilt infection centers consistently averaged 2.1 dying trees. However, the number of dead trees per infection center decreased from 4.9 in 1953 to 3.3 in 1958, and then decreased only slightly for the next 10 years, averaging 3.0 dead trees per center from 1958 to 1968 (Jeffery and Tressler 1969).

An early analysis showed that the accumulative number of oak wilt infection centers in Pennsylvania from 1956 to 1965 increased at nearly a constant rate; r = 0.098/yr (Merrill 1967). However, after 1965, the rate of new infection centers began to decrease annually. The accumulative number of infection centers in Pennsylvania from 1951 to 1971 formed a sigmoidal curve. A seventh-order polynomial regression line was a best-fit to the data, and was significant at $P < 0.0005$. The constant r value from 1956 to 1965 indicated either that the control program had no effect on the rate of establishment of new infection centers, or that there was a constant proportion of potential inoculum unaffected by the control program. After the abandonment of the control program in 1971, oak wilt did not reintensify; indeed, it continued to decline in incidence. One must conclude that the control program had no effect on the formation of new infection centers, and that the epidemic just "ran its course." Why oak wilt appeared to intensify and then subside is unknown.

In West Virginia the accumulative number of infection centers increased in a curvilinear fashion from 1956 to 1965; r = 0.315/yr (Merrill 1967). However, the epidemic curve was not truly logistic; throughout that period the r value continued to decrease, and for the last five years was 0.225/yr. From 1951 through 1965, the best-fit regression line for the data was a second-order polynomial significant at $P < 0.0005$.

Much of the early research on oak wilt in Pennsylvania involved inoculating large numbers of trees throughout the south-central portion of the state to study rate of spread, mat formation, longevity, insect vectors, and control tactics. These efforts thereby greatly increased the inoculum load in the south-central portion of the state. Far fewer oaks were inoculated in research studies from 1965 to 1968 than before, and very few trees were inoculated after 1968. It seems quite possible that the intensification of oak wilt in Pennsylvania from the early 1950s through the mid-1960s was due to the research directed towards "solving" the oak wilt "problem."

CONCLUSIONS

Results of unpublished PSU studies in Perry County had indicated that untreated oak wilt infection centers became inactive within three to five years. In 1968-69 we determined from state records that in Pennsylvania 8.6 healthy oaks were felled for every diseased oak. From a "windshield survey" we also estimated that lightning strikes were killing more oaks than oak wilt along the ridgetops and upper slopes in south-central Pennsylvania. These facts and observations, the results of the second post-control appraisal study, and the PSU analyses indicated that the Pennsylvania control program had little if any effect on the local spread and no effect on long-range spread of oak wilt.

In 1971, PaBPI transferred the control program to the PaBF which did a very limited aerial survey in 1972 using personnel lacking previous experience with oak wilt survey and detection. They found very few infection centers in comparison with those detected the previous year and thus concluded that oak wilt was not a disease of consequence and ceased all further activity. Simultaneously, in 1971 the PaBPI, which had been supporting the oak wilt research in Pennsylvania for 20 years, stated that if the state's control efforts were not worth while, then research effort on the disease also was of questionable value. Hence, they terminated all support for oak wilt research. All oak wilt research and control efforts in Pennsylvania ceased on 31 December 1971.

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Figure 1. Number of new oak wilt infections centers discovered per year in Pennsylvania, 1950 to 1971. If data from 1950-52 are omitted, the best fit line is a simple regression significant at $P < 0.0005$.

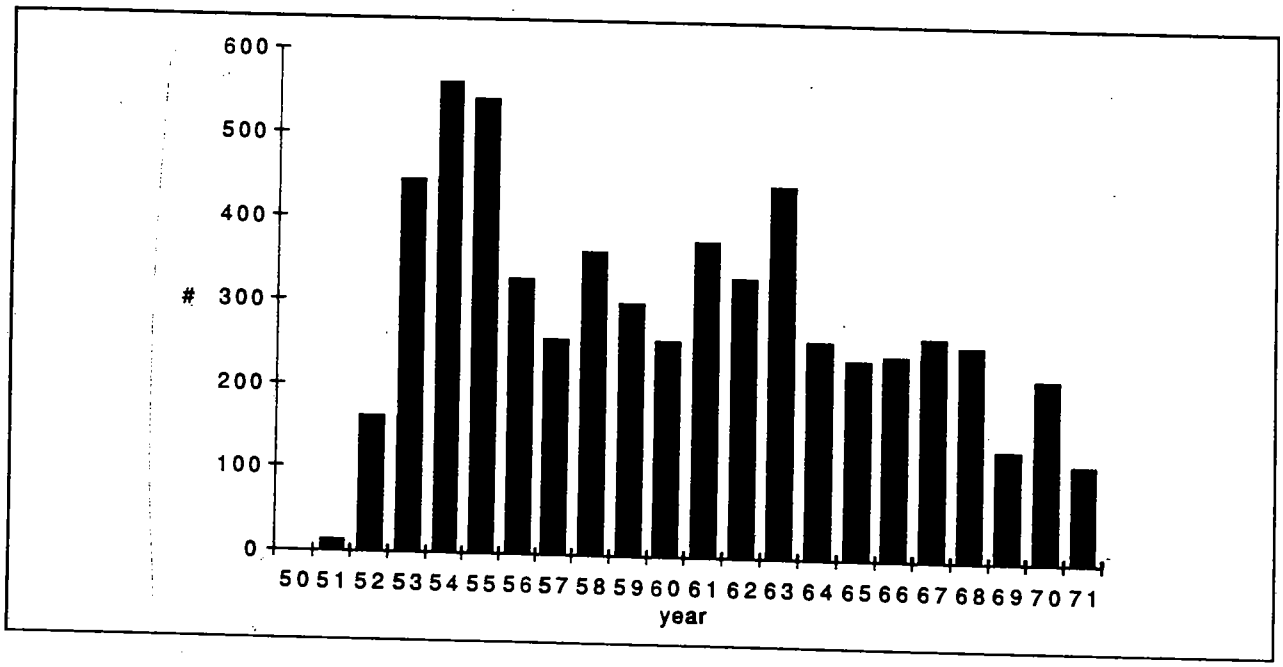
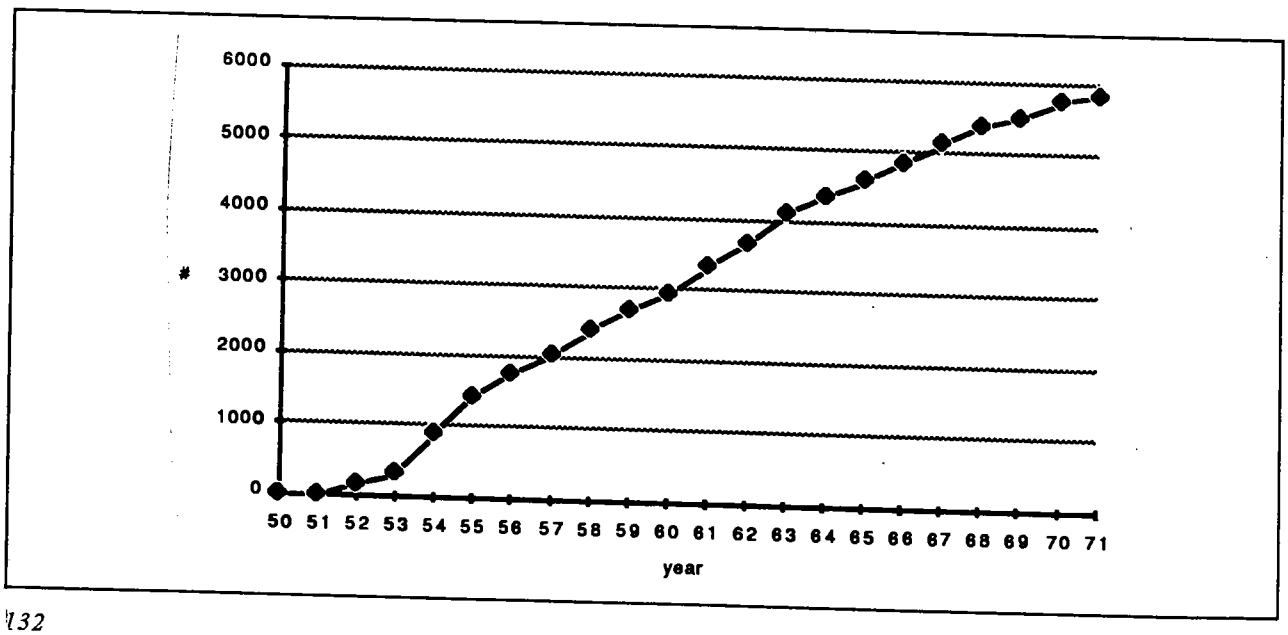


Figure 2. Cumulative number of oak wilt infection centers in Pennsylvania, 1950 to 1971. A seven-order polynomial significant at $P < 0.0005$ best fits this sigmoidal curve.



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Section IV
Oak Wilt Management in Texas

Central Texas Plant Ecology and Oak Wilt

Aaron S. Reisfield

The interplay between oak wilt, caused by the fungus *Ceratocystis fagacearum*, and the unique plant communities of central Texas is addressed. I suggest that the reason the epidemic is centered in the Hill Country is partly due to the association of live oak (*Quercus fusiformis*) and Texas oak (*Q. buckleyi*), both considered dry-adapted Edwards Plateau morphotypes of eastern entities. The potential for a disease epidemic has been realized due to the influences of European settlement. Fire suppression and the introduction of livestock has contributed to the degradation of grasslands and an increase in woody species, including vast clonal stands of live oak vulnerable to an epidemic. The low tree-species diversity has significantly affected the vector-host relationship. Local residents also promote pathogen spread, presumably by transporting infected firewood and creating infection courts. The effect of the epidemic on the endangered golden-cheeked warbler (*Dendroica chrysoparia*) is briefly addressed.

INTRODUCTION

Central Texas is a region of biological transition, a blend zone where disparate biotic elements are filtered and intermix. Geographically located in a climatic overlap zone, regions are delineated by physical features of the landscape, some with names supplied by nineteenth-century geologists (e.g., Grand Prairie, Edwards Plateau). In particular, the Edwards Plateau, a limestone-layered tableland that dips gently southeastward, contains a diversity of habitats that form a giant melting pot for vastly different biotas. Most distinctive are the steep, spring-fed canyons and narrow divides formed at the Balcones fault zone, where weathering by high-gradient streams through the upthrown region has produced the dissected (Balcones) Canyonlands. This is the famous "Hill Country" of central Texas.

Attracted by the numerous springs and other features of the Canyonlands, settlers gradually founded a rim of cities (e.g., Austin, San Antonio) along the southeastern Plateau boundary (note that Interstate Highway 35 parallels the escarpment). Today, these expanding population centers are facing the challenge of responsible environmental stewardship. The beloved sheltered canyons, artisan springs, spring-fed perennially-flowing rivers, and natural caves are now threatened. Here in Austin, efforts to protect the environment are pitted against projects to exploit it. Against this backdrop of heated and (lately) acrimonious debate, we should consider the interplay between oak wilt, caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt, and the central Texas environment. Three common questions are addressed below: 1) Why is the oak wilt epidemic in Texas centered in the Hill Country? 2) What is the human influence on the epidemic? 3) How is oak wilt likely to affect the endangered golden-cheeked warbler, *Dendroica chrysoparia*?

WHY IS THE OAK WILT EPIDEMIC CENTERED IN THE HILL COUNTRY?

To begin with, the question contains an assumption that is accurate (personal observation) and has been

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Figure 1. Natural regions of Texas.

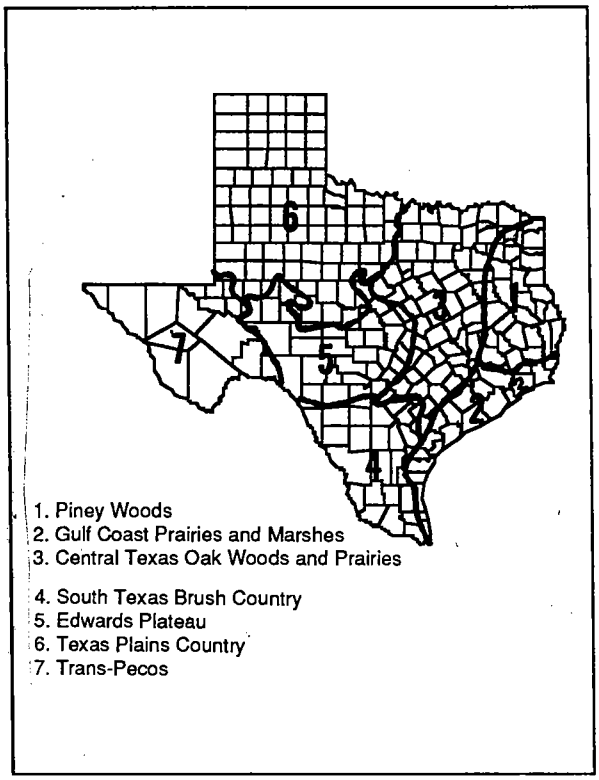
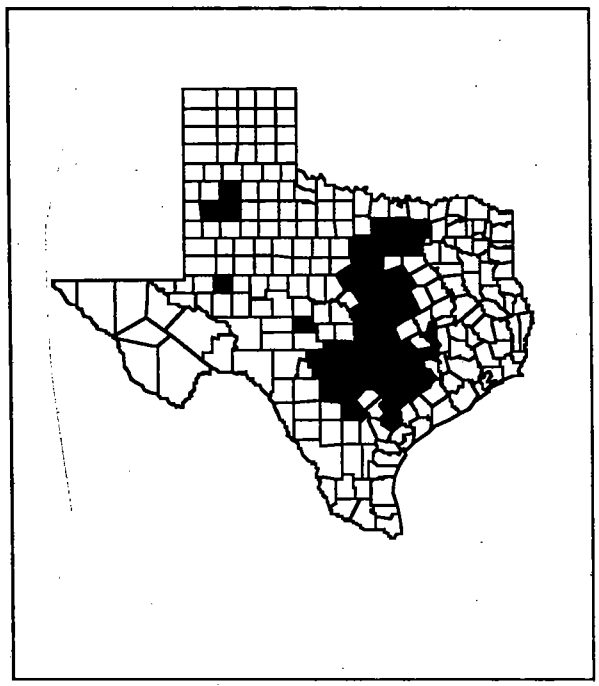


Figure 2. Distribution of oak wilt in Texas.



addressed elsewhere (Appel and Lewis 1985, Appel and Maggio 1984). From the most severely-impacted southeastern part of the Edwards Plateau, reports of the disease extend to the north and east into the central Texas Oakwoods and Praries and to a slight extent north and westward into the Texas Plains Country (Figures 1 and 2). Plant associations have been variously described for these regions (McMahan et al. 1984, Riskind and Diamond 1986, 1988), but oak-dominated communities are pervasive. Oak mortality in Texas predominantly involves live oaks (*Quercus fusiformis* Small), most of which become infected by subterranean fungal movement through tree-to-tree root connections. It is on red oaks (subgenus Erythrobalanus), however, that sporulating fungal mats may form, which may potentially attract insect vectors and promote long-distance or overland colonization of the disease organism.

To the east of the Plateau, the ranges of live oaks and several red oak species overlap, but they are rarely dominants or abundant in the same plant associations. Most red oaks prefer the sandy, low-PH soils characteristic of the Pineywoods, while the coastal live oaks prefer the heavier, fine-textured soils of the Gulf Coast Prairies and Marshes. Some hydrophytic red oaks (e.g., *Q. nigra* L., *Q. hemisphaerica* Bartr., *Q. laurifolia* Michx.) may typically be found growing near live oaks, but the plant communities are diverse (Diamond, personal communication) and the trees at least are somewhat dispersed (cf. below).

The relatively harsh climate of central Texas serves as a meeting place for several entities with eastern affinities that have dry-adapted morphotypes in the region. Trees that are large, single-bole individuals on low, moist (often eastern) sites (e.g., white ash, *Fraxinus americana* L., sugarberry, *Celtis laevigata* Willd.) may grade into shrubby forms (e.g., Texas ash, *Fraxinus texensis* (Gray) Sarg., netleaf

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Figure 3. Live oak complex in Texas.

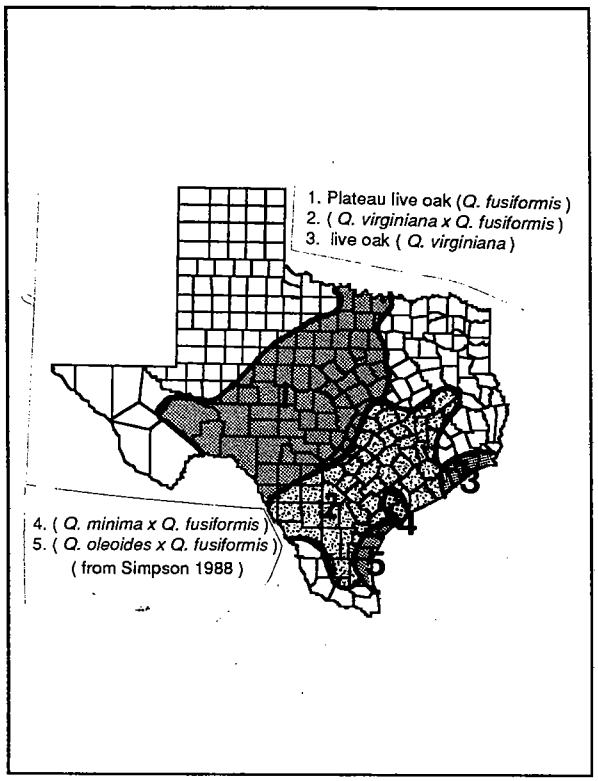
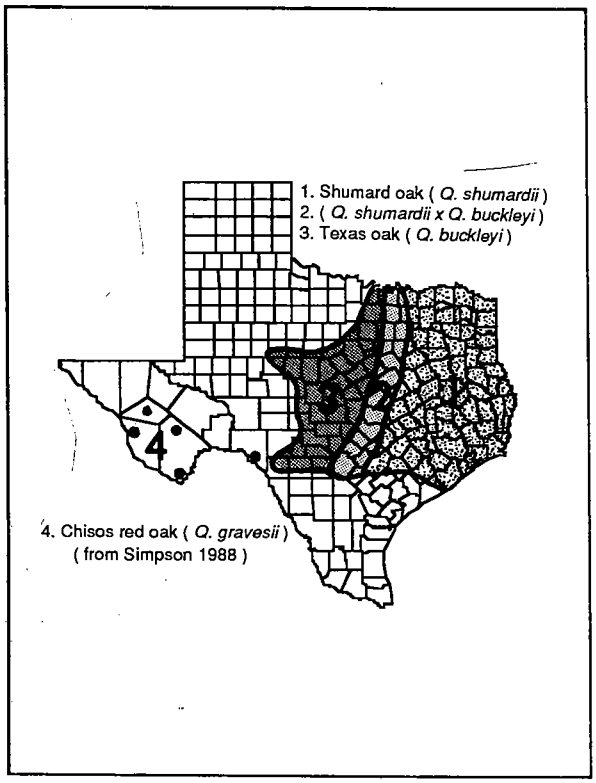


Figure 4. Red oak complex in Texas.



hackberry, *Celtis reticulata* Torr.) on upper slopes and scarp edges of the Plateau (Buechner 1944, Gehlbach 1988). So too, the live oak and red (Texas) oak (*Q. fusiformis* and *Q. buckleyi* Dorr & Nixon) so pervasive in the Hill Country are xerophytic-adapted extensions of entities (*Q. virginiana* Mill. and *Q. shumardii* Buckl.) with eastern affinities (Simpson 1988, see Figures 3 and 4). These entities grow in different plant associations to the east, but are brought together in the Hill Country. The distributional area shared by the two Plateau morphotypes is highly correlated with the current distribution of oak wilt.

In the Hill Country, floodplain gallery woodlands or forests may contain both live and Texas oak growing alongside elm and hackberry. On mesic, north or east-facing steep slopes, deciduous woodlands or forests also often contain both oak species. On drier, southern or western exposures, live oak may be co-dominant with Ashe juniper (*Juniperus ashei* Buchh.), forming evergreen woodlands that may also include Texas oak (to the west, these woodlands are restricted to north and east-facing exposures). Grasslands found on (undisturbed) uplands and broad valleys also may contain oaks as scattered individuals or in motts, creating a savanna or park-like appearance. The northern part of the Plateau (the Lampasas Cut Plain) is flatter and more open, but otherwise similar to the Hill Country (Riskind and Diamond 1986, 1988).

Thus, both live oak and Texas oak are represented in most Hill Country plant associations. Furthermore, these are common and often dominant species. In contrast, live oak and red oak trees in eastern Texas are typically more widely dispersed (Simpson 1988). In the Hill Country, even when the two species grow in distinct plant associations, they may be in close proximity due to the dissected

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landscape (e.g., a south vs. north-facing steep slope). Also, these factors are compounded by the lower tree-species diversity in the Hill Country, a critical factor with regard to vector-host relationships (i.e. the likelihood of an insect vector visiting both oak species). Community structure and diversity should not be underestimated, especially since overland disease transmission is an inefficient process, occurring only within a narrow range of conditions (D. N. Appel, personal communication).

Where the plant associations described above disappear abruptly, as across the Balcones Escarpment, there is also a dramatic decrease in disease incidence. (This fact is obscured by the oak wilt distribution map, shown in Figure 2, which does not discriminate between few or many reports in a given county). However, to the north and west of the Hill Country, as the land becomes less dissected and the climate more arid, grasslands gradually become more extensive and the distributional limit of oak wilt is less marked. There, live oak is gradually replaced by mesquite (*Prosopis glandulosa* Torr.) which favors the flatter, heavier soils. Texas oak, too, becomes less common to the west, with Lacey oak (*Q. laceyi* Small) becoming important on the southern margin of the Plateau. Post and blackjack oaks (*Q. stellata* Wang., *Q. marilandica* Muenchh.) are more important in the far north. Note that currently, the western limit of oak wilt coincides with the 28 in. average annual precipitation isoline (Figure 5). Also, oak wilt has never been reported from the granitic Llano Uplift, despite the fact that live oak is a major component there. It is significant that Texas oak (as well as the ubiquitous Ashe juniper) is nearly absent from the sandier, low-pH soils of this region (Riskind and Diamond 1986).

WHAT IS THE INFLUENCE OF HUMANS ON THE OAK WILT EPIDEMIC?

Above, I've explained that the association of live oak with red oak in the Hill Country has created the potential for disease outbreak. However, there is no evidence, in the literature or elsewhere, that there was widespread oak mortality in Texas before the middle of this century (D. N. Appel, personal communication). It is now clear that the influence of European settlement is largely responsible for the catastrophic losses currently being sustained in central Texas. A comparison of Figures 2 and 6 illustrates the homology between human population increase and the distribution of oak wilt in the Hill Country (Figure 6 represents 1960-1970; 1950-1960 was similar).

The influence of settlement is of three basic types; 1) the introduction of domestic livestock and suppression of recurring wildfires has led to a degradation of the grasslands and an increase in woody species, respectively - these activities have fostered development of vast, homogeneous stands of live oak vulnerable to a disease epidemic, 2) infection courts (wounds) are created on host trees in various ways as lands are developed, and 3) infected oaks cut for firewood are transported away from disease centers, often into areas without previous record of the disease, but with a vulnerable host population (such as a homestead or urban center, precisely where tree wounds are common place). As the focus of this paper is plant ecology, I will address only type 1 below.

Historical descriptions of Hill Country uplands emphasized grassland communities (Bray 1904, Buechner 1944), but woodlands and forests also were reported by early travelers, apparently limited to rocky hillsides, eroded canyons, and creek or river-banks (Van Auken 1988, Weniger 1988). Grasslands were pervasive on the deeper upland soils, where fires were more frequent. Occasional live oaks grew on the uplands, as single trees or in motts (Amos and Gehlbach 1988).

Settlement of the Hill Country began in the 1840's, as large scale German immigration moved from east to west (Palmer 1986). Cattle were introduced about 1860, followed by sheep and goats. Before definite boundaries of ownership were established, while "free grass" was still available, Buechner (1944) writes of the "disastrous overstocking" from which central Texas rangelands have never recovered. The heavy sod covering was broken up by overgrazing, leading to erosion, and allowing invasive woody species to become established.

Before European settlement, prairie-fires occurred periodically, ignited by lightning, American Indians,

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Figure 5. Average annual precipitation in inches.

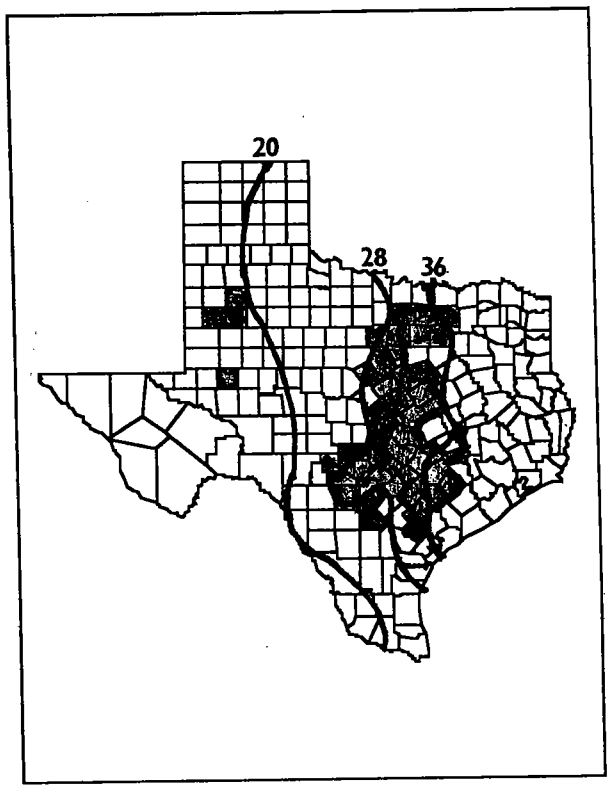
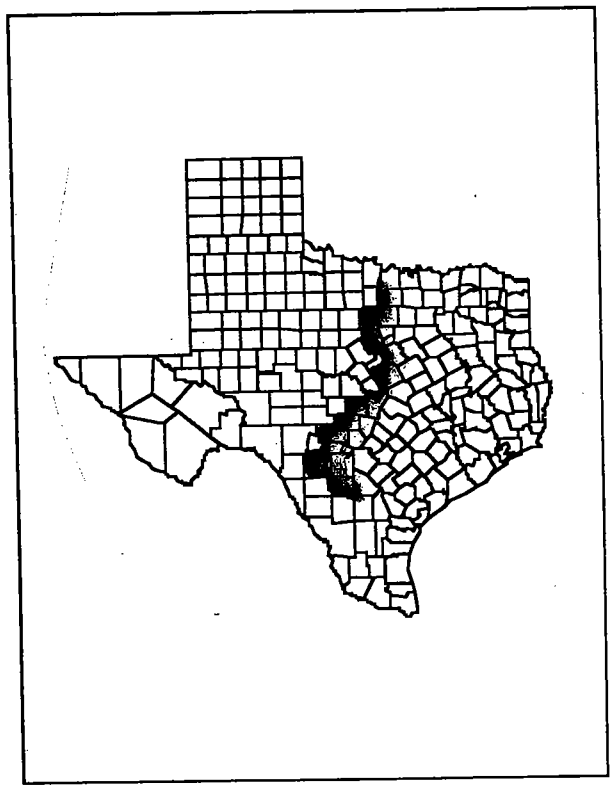


Figure 6. Population increase in central Texas counties.



or both (Fonteyn et al. 1988). Afterwards, fire suppression promoted the expansion of woody plants at the expense of grasses. Also, fire frequency decreased when overgrazing livestock removed the light, fluffy, dry-grass fuel (Van Auken 1988).

Upon degradation of the grasslands, various woody species have exhibited unique patterns of recruitment into this community. The dramatic increase in density of Ashe juniper (locally called cedar) in this century has been the subject of much discussion and research (see Amos and Gehlbach 1988). The opportunistic juniper differs from live oak in being berry-dispersed, extremely fire-sensitive, and a low preference for browsing deer and livestock. Today, with fire at a minimum, it is an aggressive invader of overgrazed grasslands. Unlike juniper, live oak and mesquite are considered natural components of upland tallgrass and shortgrass communities, respectively (Riskind and Diamond 1988). While juniper berries have been disseminated by livestock and wildlife, live oaks have been colonizing new territories by means of lateral root growth and root suckering. The live oak's adaptive success involves its propensity to regenerate in this way, and to maintain vast subterranean networks of roots that connect trees to each other (Appel and Lewis 1985, Muller 1951).

The invasive live oak and juniper now dominate evergreen woodlands that are continuous with grasslands throughout central Texas. Various authors have discussed the structure of these communities. Fonteyne et al. (1988) suggested that winter fires historically promoted live oak-juniper associations (fire-sensitive junipers were protected to a degree beneath canopies of cool-burning live oaks), while summer fires would promote a savanna dotted with live oak only. Buechner (1944) proposed a

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mechanism for the formation of live oak motts of various sizes. When foliage is eliminated from the crown of a young tree by browsing, the tree's food reserves are utilized in reproduction by root suckers. When trees grow large enough to escape browsing, their canopies eventually shade out grasses below, and subsequent grazing and browsing may eliminate additional root sprouts.

Mesquite and juniper are viewed by ranchers as nuisance weeds and have been perpetual targets of various means of chemical and mechanical brush control. The proliferation of live oak, on the other hand, has been viewed with favor by Central Texans who have encouraged its spread. This beloved tree is cherished by ranchers and city-dwellers alike for a host of utilitarian and sentimental reasons. Also, because of its short, massive bole and twisted limbs, its industrial value is limited, and commercial harvest has been avoided.

The degradation of the original grasslands combined with selective removal of other woody species has enabled the live oak to form vast, homogeneous, clonal populations in species-poor plant associations. Although authors disagree as to whether genetic uniformity *per se* always acts to exacerbate disease epidemics (Parker 1988), the clonal population structure of live oaks has done this since interconnected roots act to convey the pathogen from tree to tree. The rhizomaceous habit is compounded by the likelihood of grafting between roots pressing tightly against each other in restrictive fractures characteristic of Plateau limestones (Appel et al. 1989). A similar situation has been described for Wisconsin and Minnesota, where stands of northern pin oak (*Q. ellipsoidalis* E. J. Hill) continue to incur dramatic losses to oak wilt. There, fire and extensive logging are cited as having destroyed the original, diverse woodland (Gibbs and French 1980).

HOW IS THE OAK WILT EPIDEMIC LIKELY TO AFFECT THE GOLDEN-CHEEKED WARBLER?

From an economic and aesthetic viewpoint, the effects of oak wilt are potentially devastating, and those who dwell in hard-hit localities can already testify to this. The effect of oak wilt on the region's ecological dynamic is more complex and difficult to assess. With the issue of endangered species in the limelight of local politics, the possibility of habitat degradation should be examined.

The endangered golden-cheeked warbler (GCW) winters in southern Mexico and Central America, but its summer breeding range is confined to central Texas, where it prefers mature oak-juniper woodlands in dissected terrain such as canyons and ravines (Butler/EH&A Team 1990). Although the oak wilt epidemic may be viewed (in a sense) as an ecological check on the increasing density of live oak in central Texas, its immediate impact on warbler habitat is probably a negative one, since woodland and forest canopies are opened as the oaks die. After thousands of live and red oaks died at the Kerrville State Recreation Area, Wahl et al. (1990) found no warblers in this area, even though they were known to previously breed there. On the other hand, in no way does the disease promote a primeval grassland; rather, invasive species such as Ashe juniper tend to proliferate when the live oaks die (personal observation).

Since the steeper Hill Country slopes have probably always been heavily wooded with oaks, it would be incorrect to view the disease as promoting a condition of ecological equilibrium. The long term effects of oak wilt, however, are not clear. Oak regeneration after the pathogen has moved through an area is not well understood, and the degree of success oak wilt suppression efforts will yield on an area-wide basis remains to be seen. Hopefully, efforts to preserve large tracts of critical habitat will be successful, and an integrated approach to oak wilt suppression where feasible is being recommended.

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The Texas Oak Wilt Suppression Project:
Development, Implementation, & Progress To Date

R. Scott Cameron
Ronald F. Billings

With financial support from the USDA Forest Service, the Texas Forest Service initiated a cooperative suppression project in 1988 to address a severe oak wilt problem in Texas. The primary objectives of the project were; 1) to promote public awareness of oak wilt prevention and control and 2) to detect, evaluate and control individual oak wilt infection centers and sources of inoculum within designated areas of operation. The project initially was limited primarily to nine counties in central Texas. A full-time project staff was hired, trained, and set up in key locations within the affected area. A technical advisory board, technical guidelines, two project circulars, a slide series, a mobile display on oak wilt, and a computer-based informational system have been created. During the four years since the project began, 1,122 oak wilt centers have been detected, over 350 centers have been treated with more than 100 miles of trenches to stop further spread of the disease, 1,190 diseased red oaks have been eliminated, and over 1,700 high value trees have been treated with fungicide. Landowners have been reimbursed for up to 50% of the costs of approved treatments. Post-suppression evaluations have been conducted at least once per year to document the efficacy of those project treatments in which trenches were included.

INTRODUCTION

In the central Texas landscape, stately live oak trees (*Quercus virginiana* Mill. and *Q. fusiformis* Small) are highly regarded for their beauty, shade, and forage for wildlife. Many of these live oaks have been the focal points for historical events (Texas Forest Service 1984). Widespread mortality among these highly-valued live oaks in central Texas has been recognized for many years (Dunlap and Harrison 1949, Taubenhaus 1934, Van Arsdel and Halliwell 1970) and oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, was officially diagnosed in Dallas in 1961 (Dooling 1961). But it was not until the late 1970's that this widespread mortality of oaks in central Texas was attributed to the oak wilt pathogen (Lewis and Oliveria 1979). This realization sparked interest in research on this disease in Texas and provided the impetus for two important cooperative projects initiated in the 1980s; the "Oak Wilt Demonstration Project" and the "Oak Wilt Suppression Project."

TEXAS OAK WILT DEMONSTRATION PROJECT

The Texas Oak Wilt Demonstration Project was carried out from 1982-1987 to evaluate the behavior and extent of the oak wilt problem in central Texas. This project was funded by the USDA Forest Service, Forest Pest Management, and was jointly administered by the Texas Forest Service (TFS) and the Texas Agricultural Experiment Station. Accomplishments of this demonstration project and

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other associated research projects have been substantial; 1) distribution and severity of oak mortality was documented in three areas in central Texas using color-infrared aerial photography (Appel and Maggio 1984), 2) the confirmed distribution of oak wilt in Texas was expanded from a few to over 30 counties, 3) the rate of expansion of selected oak wilt centers in live oak stands was measured using sequential aerial photography (Appel et al. 1989), 4) techniques for diagnosing oak wilt in live oaks were improved (Appel 1986, Lewis 1987), 5) nitidulid (sap feeding) beetles were implicated as vectors of oak wilt in Texas (Appel et al. 1986), 6) trenching and tree removal were pilot tested for oak wilt control in Texas (Appel and Lewis 1985), and 7) the potential for reforestation in oak wilt centers using live oak and alternate tree species was evaluated in several locations in central Texas (Texas Forest Service, unpublished data).

The knowledge and experience gained from five years of the Texas Oak Wilt Demonstration Project, together with the extensive literature on oak wilt control (Himelick and Fox 1961, True and Gillespie 1961, Jones 1965, 1971, French and Stienstra 1980, Appel and Lewis 1985; Johnson and Horne 1986), provided the foundation for the proposal of a Texas Cooperative Oak Wilt Suppression Project in 1988. This paper will describe the development and implementation of this suppression project and briefly summarize the accomplishments for the first four years.

DEVELOPMENT OF THE TEXAS OAK WILT SUPPRESSION PROJECT

Project Proposal

The Forest Pest Control Section of the Texas Forest Service developed an Oak Wilt Suppression Project proposal and submitted it to the USDA Forest Service, Forest Pest Management, in September 1987. This proposal consisted of a biological evaluation, a cooperative oak wilt control plan, and an environmental analysis. In compliance with the National Environmental Policy Act (NEPA), the "scoping process" was followed to examine the proposed suppression action and its possible effects on the environment, seek public involvement and critique of proposed alternatives, identify pertinent issues, and identify the responsible official and cooperating agencies.

In June 1988, the USDA Forest Service Southern Regional Forester issued a "Decision Notice and Finding of No Significant Impact" and the project was officially approved for a five-year period. To address possible new impacts on the project brought on by the 24 (c) "special local need" registration of the fungicide propiconazole (Alamo®) for oak wilt treatment in 1990 and the addition of the golden-cheeked warbler, *Dendroica chrysoparia*, to the endangered species list, a supplement to the original environmental assessment was prepared and approved in August 1990.

Technical Advisory Board

To provide project guidance and direction, a Technical Advisory Board was formed, consisting of key administrators and specialists with the USDA Forest Service, Texas Forest Service, Texas Agricultural Experiment Station, Texas Agricultural Extension Service, the City of Austin, and a private tree care company. This advisory board first met in December 1987 to discuss the project proposal and implementation process. Since then, it has met annually to review project activities and accomplishments and provide long-term direction.

Objectives

The primary goal of the Texas Cooperative Oak Wilt Suppression Project is to minimize the spread of oak wilt in selected rural and urban areas of central Texas and within the City of Austin. Specific objectives of the Project are to; 1) establish a full-time coordinator and trained field staff in key areas within central Texas to implement the Project, 2) initiate and accelerate public awareness campaigns to educate urban and rural landowners of the oak wilt threat as well as prevention and suppression alternatives, 3) identify oak wilt centers in selected suppression areas using aerial surveys and contacts with local landowners, 4) provide ground verification and diagnosis of oak wilt infection centers located

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through detection surveys and other sources, 5) notify landowners of oak wilt centers in target counties and provide control recommendations, 6) assist with implementation of control treatments by providing technical assistance and cost-share funds for approved treatments, 7) conduct post-suppression evaluations by periodically revisiting all infection centers treated with project funds to record breakouts and assist with retreatments if necessary, and 8) develop and refine a computerized record keeping system for cataloguing and summarizing detection, ground evaluation, and control information.

Organization

Prior to the initiation of this project, the Texas Forest Service did not routinely operate in the central Texas area, except for the activities of two urban foresters located in Ft. Worth and San Antonio. Indeed, before the Oak Wilt Demonstration Project was carried out in the 1980s, the enormous impact of this disease and the large area affected by oak wilt was not widely recognized. Thus in 1988, project activities were initiated in Hood, Travis, and Kendall counties, and within the City of Austin. Gradually, the area of operation has been increased to include a total of nine target counties with the addition of Erath, Bosque, Hays, Williamson, Gillespie, and Bandera counties. Oak wilt infection centers occasionally have been treated with project funds in about 12 additional counties.

The Project is headed by a project director at the TFS headquarters in College Station, Texas. The project director is assisted by administrative and technical coordinators with the TFS Forest Pest Control Section in Lufkin, Texas. Field personnel gradually have been added to the project to carry out specific project objectives and to address increasing numbers of requests for assistance in key counties in central Texas. Currently, the field staff for the Project consists of a field coordinator based in Austin and three project specialist/foresters in Austin, Meridian, and Kerrville and one technician in Granbury/Ft. Worth.

In addition, two TFS urban foresters have contributed up to 30% of their time toward implementing the Project. The City of Austin has a full-time oak wilt forester and a technician to implement project objectives. In most years, seasonal technicians have been employed as needed during summer months to assist full-time project personnel in central Texas. Support duties are provided by the TFS secretarial staff in Lufkin and College Station.

Funding

Federal funding (50%) for this cooperative suppression project is being provided by the USDA Forest Service, Forest Pest Management, Atlanta, Georgia. Matching funds are provided by the Texas Forest Service (32%), the City of Austin (3%), and private landowners in central Texas (15%). Other organizations assisting with this project include the Texas Agricultural Extension Service, Texas Agricultural Experiment Station, Soil Conservation Service, Agricultural Research Service, and Travis County. Federal funds allocated annually for this project were $169,000, $345,000, $345,000, $466,000, and $450,000 from 1988 through 1992, respectively. Including the State, City of Austin, and private landowner matching contributions, the total expenditure for this suppression project has leveled out at about $900,000 per year.

Control Tactics

The Texas Oak Wilt Suppression Project has a two-faceted approach to oak wilt management -- prevention and direct control. Prevention is promoted through public education on proper timing of pruning and treating wounds on oak trees, elimination of potential red oak inoculum sources by destroying diseased red oaks, proper handling of firewood, use of propiconazole fungicide, and planting diverse and resistant tree species. Direct control procedures include detection, field evaluation, and control of individual oak wilt centers. Through a cost-share program landowners are reimbursed for up to 50% of their expenses for treatments approved by the Project Director prior to installation.

Approved control procedures include installation of trenches, at least three-ft deep, to prevent tree-to-tree spread of the fungus through interconnected root systems. A variety of equipment can be used to

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Table 1. Texas Oak Wilt Suppression Project Accomplishments, June 1988 through May 1992.

Federal Fiscal YR	Centers Detected	Centers Trenched	Feet of Trench	Red Oaks Removed	Trees Injected
1988	362	14	19,533	74	0
1989	168	42	61,065	53	0
1990	184	54	65,779	11	77
1991	255	154	270,890	414	739
1992	153	86	158,936	638	886
Totals[1]	1,122	350	576,203	1,190	1,702

[1] Totals through May 31, 1992

install trenches, including rotary rock saws, belt trenchers, back hoes, and ripper bars. Rock saws are most often used in urban areas and ripper bars pulled by bulldozers are more commonly used in rural areas. Initially, trenches were installed 75 feet in advance of the most recently symptomatic trees, but results of post-suppression evaluations indicated that this distance was not sufficient to provide effective control in some cases. Current recommendations call for placement of trenches 100 ft in front of symptomatic trees. At least one apparently healthy "buffer" tree should be included between symptomatic trees and the trench.

Whenever practical, especially in rural areas, it is recommended to up-root and dispose of apparently healthy trees in the buffer between symptomatic trees and the trench. Originally, recommendations allowed for use of herbicides to deaden diseased and apparently healthy buffer trees without use of a trench. However, a research project conducted by the TFS (Barber et al. 1990) clearly demonstrated that triclopyr herbicide did not induce rapid mortality of stumps and root systems, and cut-stump treatments occasionally killed adjacent healthy trees. Thus, herbicide treatments are no longer recommended except for treatment of diseased red oaks to hasten the drying process, thereby reducing the probability of fungal mat formation. Cost-share funds also can be used for the removal and disposal of symptomatic red oak trees to prevent fungal mat formation.

Propiconazole prevents many trees challenged by the pathogen from developing severe disease symptoms (Appel and Kurdyla 1992), but it does not prevent the pathogen from moving through the untreated root systems and continuing through a stand of live oaks. Also, retreatments may be necessary, especially with these therapeutic treatments where trees already are infected at time of treatment. Therefore, the primary justification for incorporating propiconazole treatments in the Texas Oak Wilt Suppression Project is to provide landowners an incentive to cooperate in trenching operations designed to stop the spread of the disease. Cost-share funds can be used to apply propiconazole (Alamo®) solely to high-value, non-symptomatic trees inside cost-shared trenches. Considerable quantities of propiconazole provided free of charge by Ciba-Geigy Company have been distributed by the TFS to treat high-value trees inside cost-shared trenches.

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Technical Guidelines

A loose-leaf manual containing operational policies and technical guidelines was prepared and distributed to project personnel. The operational policies primarily address project development and cost-share procedures for internal use. The technical guidelines were prepared with the assistance of Dr. David Appel, Texas A&M University, and Dale Starkey, USDA Forest Service, Forest Pest Management, and approved by the Technical Advisory Board.

These technical guidelines were designed to be easily revised as necessary and copied to provide land owners with additional information on oak wilt treatments. Technical guidelines currently in the manual include recommended procedures for; 1) detection and ground evaluation, 2) field and laboratory diagnosis, 3) root disruption techniques, 4) tree removal and disposal, 5) red oak treatment, 6) fungicide (Alamo®) treatment, 7) firewood use, 8) avoiding effects on the endangered golden-cheeked warbler, and 9) post-suppression evaluation.

Oak Wilt Information System

To track project activities and accomplishments, the Texas Forest Service designed and implemented a computerized record-keeping system (Oak Wilt Information System - OWIS). This record-keeping system was written in D-Base III for IBM-compatible microcomputers by TFS Staff Forester Roger Lord. Project personnel input data on personal computers at each field station. They can access their records at any time to keep track of treatment status and detailed treatment information on individual infection centers. Current data are periodically sent from each field station via electronic mail or diskette to the Forest Pest Control Laboratory were the master records are maintained. Through a series of pre-programmed reports, project administrators have ready access to data summaries for use in periodic reports, post-suppression evaluations, or economic analyses.

PROJECT IMPLEMENTATION AND ACCOMPLISHMENTS

Public Awareness of Oak Wilt

Project personnel are continually involved in efforts to make central Texas landowners aware of the oak wilt problem and available methods of diagnosis, control, and prevention. These efforts can be categorized as public presentations on oak wilt, media events, and individual on-site landowner assists. During the four-year period from June 1, 1988 through May 31, 1992, project personnel carried out a total of over 350 public presentations, 100 media events, and 2,000 on-site landowner assists. Also, many more landowners have been assisted through undocumented telephone calls.

To further promote public awareness of oak wilt and the Suppression Project, two circulars were published and widely distributed to interested landowners and neighborhood groups. These are titled "How to Identify and Manage Oak Wilt in Texas" (Appel et al. 1990) and "Save Our Shade - A Guide to Cost-Sharing for Oak Wilt Control in Texas" (Texas Forest Service 1990). Also, a 35 mm slide series and a portable photo display describing oak wilt and Project activities have been prepared for public presentations.

Project personnel, in cooperation with Dr. David Appel, organized and hosted the National Oak Wilt Symposium in Austin at which this paper was presented. Project accomplishments were highlighted in the Symposium field trip and in this and several other technical presentations. In further recognition of the importance of oak wilt in Texas, Ann Richards, the governor of Texas, declared the week of June 22-28, 1992, as "Oak Wilt Awareness Week."

Identification and Confirmation of Oak Wilt Centers

Detection of oak wilt centers by Project personnel is achieved by conducting aerial survey flights over predetermined areas or by responding to landowner inquiries. Intensive aerial surveys and ground verification were conducted in Hood and Kendall counties during the first two years of the project and

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in Bosque County starting in 1990. Most of the other new oak wilt centers were detected as a result of landowner inquiries. During the four-year period from June 1988 to June 1992, a total of 1,122 oak wilt centers were detected and confirmed by project personnel (Table 1). The majority of these centers were diagnosed by noting the presence of symptomatic leaves (veinal necrosis) on live oaks. However, positive identifications were obtained through laboratory culture at Texas A&M University for many of the oak wilt centers for which cost-shared treatments were installed.

Control Treatments

Project specialists work with individual landowners or neighborhood groups to identify the location of oak wilt center boundaries. If the infection center is well defined and considered containable, and the landowner is willing to implement the suggested control treatment, the project specialist prepares a written oak wilt suppression plan. The plan, together with an estimate of costs and a request for cost-shares, is submitted for approval to the Project Director. If the treatment plan is approved, the treatment is installed under supervision of the Project specialist. After the treatment is completed, the landowner or neighborhood organization is reimbursed with federal funds for up to 50% of the treatment costs.

A summary of cost-shared suppression treatments utilizing trenches for the period June 1988 through May 1992 is presented in Table 1. A total of 350 oak wilt centers have been treated with trenches with annual accomplishments steadily increasing through the first four years as the Project has grown in personnel and experience.

Through October 1, 1991, a total of about $640,000 had been spent on cost-shares. The majority (64%) of these funds have be used for trenching, the primary means of attack for halting the local spread of individual oak wilt centers in live oak stands. Other treatments receiving cost-share funds include tree cutting (16%), tree pushing (6%), tree removal (4%), and fungicide treatments (10%).

Land Owner Participation: Community Organization

One unique aspect of this suppression project is the involvement of multiple owners in many of the treatments, especially in urban areas. In cases where an oak wilt center is located solely on one landowner's property, project personnel usually deal directly with the property owner or a ranch foreman. In this case, procedures are relatively straightforward and treatments are usually installed quickly. However, when oak wilt centers occur in residential areas involving multiple landowners with varying interests, income levels, and values, the process is much more complicated and time consuming.

A common series of phases endured by individuals and communities planning to install an oak wilt treatment include; education, realization, despair, determination, cooperation, mobilization, and finally implementation. It is inefficient for the few Project personnel to work one-on-one with each person in the neighborhood. Thus, Project specialists generally meet first with neighborhood groups or interested individuals to familiarize them with the problem and the steps that can be taken to halt the advance of the disease. Then certain particularly motivated neighborhood and community leaders usually take charge and spend countless hours contacting neighbors, educating, organizing, and raising funds. Once all this preparation is completed, the neighborhood is ready for the assistance from the Project specialist to help complete the control treatments.

Various methods of raising funds to pay for oak wilt treatments have been used, including; the collection of substantial personal contributions from concerned neighborhood residents, the use of community funds to pay for the other half of treatment costs, and numerous fund-raising events such as bake sales or festivals. A few examples of the community organizations that have been formulated specifically to combat oak wilt are; Save the Oaks Fund, Inc. (SOFI) - the Live Oak Festival, Anderson Mill Oak Wilt Watchers, Edgemont Lovers, Austin Lake Estates Residents Against Oak Wilt, Walnut Creek Neighborhood Association, Hays County - Stamp Out Oak Wilt Campaign (organized

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by the Texas Agricultural Extension Service), and Medina Community Development Association - Oak Wilt Busters.

Another important community action in response to the oak wilt problem in central Texas is the passing of city ordinances aimed at stemming the spread of oak wilt. For instance, the City of Austin, the Village of Lakeway, and the City of West Lake Hills, among others, have passed ordinances which require property owners to remove and dispose of diseased trees and infected wood officially declared to be public nuisances, such as red oaks with oak wilt.

CONCLUSIONS

The Texas Oak Wilt Suppression Project is unique among pest suppression projects in that it was initiated by the USDA Forest Service and the Texas Forest Service in a region (central Texas) where neither agency previously had a strong presence. Also, it is a program designed to suppress a devastating disease for which little hope was given for successful control. However, in the four years since this project began, a professional staff has been established and is assisting private landowners over an extensive and expanding area in central Texas with education on oak wilt, detection and evaluation of infection centers, and implementation of control treatments with the assistance of cost-share funds.

The success of Project-sponsored treatments and the benefit:cost of these treatments are not addressed in this paper as these are the subjects of subsequent presentations in this symposium. However, accomplishments to date have been substantial. Perhaps most importantly, central Texas landowners are becoming more educated on oak wilt and how to cope with this devastating disease. Treatments installed as a result of this project, although not 100 percent effective, have provided hope to many desperate landowners and saved thousands of valuable oaks trees which would otherwise have been destroyed by oak wilt.

ACKNOWLEDGMENTS

The authors would like to take this opportunity to recognize and express their gratitude to the many people and organizations which have generously contributed their time, energies, and resources toward making this suppression project a success. We especially thank Congressman J. "Jake" Pickle, the USDA Forest Service, the City of Austin, and the Texas Forest Service for their continued support in obtaining and providing funding for this project; Travis County and the USDA Agriculture Research Service for providing office facilities; and the Texas Agricultural Extension Service and the Texas Agricultural Experiment Station for their continued technical support. The highly qualified and motivated field and office staff of the Texas Forest Service and the City of Austin deserve special recognition and thanks. Finally, we would like to recognize the many private individuals who have contributed countless hours and personal resources toward extending public education efforts and making it possible to establish the numerous control treatments credited to this Project.

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Evaluation of Suppression Project Treatments

E. H. Gehring

Trenching around expanding oak wilt centers is the cornerstone of the Texas Oak Wilt Suppression Project. This review compares the post suppression evaluations (PSE) of 236 trench barriers installed between 1988 and 1991 to determine their efficacy. The centers were compared by the calendar year of trench installation, time after installation to breakout, breakouts per 1,000 ft of trench, month of treatment installation, land use category, and type of trenching equipment. Overall, only 48 of the 236 trenches had breakouts (oak wilt infections immediately outside the trench) at the time of the last PSE. Fewer centers treated in 1990 (18%) and 1991 (8%) had a breakout as compared to centers treated in 1988 (80%) or 1989 (42%). The majority of the breakouts occurred within 12 mo of treatment installation. Fewer breakouts were observed for trenches installed in 1991 per 1,000 ft when compared to previous years at six-mo intervals. Trenches installed during the months of April and May were the most effective. Rural trenches installed with a bulldozer and ripper bar have had fewer breakouts to date than urban trenches installed with a rocksaw.

INTRODUCTION

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, is a devastating disease of oak trees in both urban and rural settings. The Texas Oak Wilt Suppression Project (hereafter referred to as the Project) is a concerted, cooperative effort to suppress this disease in central Texas (see Cameron and Billings, these proceedings). Since the Project began in 1988, Project personnel have identified over 1,000 oak wilt centers and treated over 650 of them. This paper reviews the results of the first four years of the Project. The data is operational, not research-oriented, and any inferences are not necessarily statistically sound. The purpose of this review is to examine how well the direct control recommendations of the Texas Oak Wilt Suppression Project are working.

This review provides the first opportunity to evaluate an operational control program for oak wilt on live oaks in Texas. In several northeastern states, oak wilt control programs have been tried and evaluated with varying degrees of success (Himelick and Fox 1961, Jones 1965 1971). All of the states employed some type of aerial detection with ground checking, while controls consisted of felling, herbiciding, girdling, or some combination thereof. The Pennsylvania barrier method consisted of felling and treating diseased oaks and surrounding trees of like species with herbicides. This barrier control method was effective but a high number of healthy trees were killed (Jones 1971). The West Virginia "deep-girdle" method did not require killing healthy trees but gave very inconsistent results (Jones 1971).

Mechanically severing root connections has been recommended for years (Himelick and Fox 1961). Trenching as a barrier method is still being recommended in Minnesota (French and Stienstra 1980, French and Cook, personal communication). One of the primary objectives of the control programs in other states was to reduce the incidence of oak wilt (MacDonald and Hindal 1981). Trenching and other

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barriers to root graft transmission can effectively halt the "local" spread of oak wilt, but do little to lower the incidence of new oak wilt centers. The Pennsylvania oak wilt control program had little impact on the number of new centers that developed (see Merrill, these proceedings).

The overall goal of the Texas Oak Wilt Suppression Project is to minimize the spread of oak wilt. This is being accomplished by increasing public awareness, identifying oak wilt centers, and cost-sharing control treatments within selected counties of central Texas. Several recommended treatments have been cost-shared through the Project, including red oak removals, fungicide treatments, silvicide barriers, rogue barriers, and trenching.

Diseased red oaks are removed to lower the potential inoculum level in the environment. As part of the Project, more than 1,000 diseased red oaks have been removed and destroyed before fungal mats could form and become sources of inoculum. Currently, there is no feasible way to measure the *C. fagacearum* inoculum level in the environment, so the effect of removing a diseased red oak cannot be determined.

In the spring of 1990, the fungicide Alamo® (propiconazole) was registered by the Environmental Protection Agency to be injected into live oaks in Texas. Shortly thereafter, fungicide injections were incorporated into Project guidelines. All of the trees injected with Alamo® as part of the Project were asymptomatic live oaks on the inside of containment trenches. Since it is too early to tell how effective this treatment recommendation will prove to be, fungicide injections are not included in the present evaluation.

Silvicide barriers installed early in the Project did not halt expanding oak wilt centers as expected. They are no longer recommended except to hasten the death and drying out of red oaks that cannot be removed and destroyed in a timely manner. Although the intended purpose of silvicides was to kill the root system, none tested was effective in halting the spread of *C. fagacearum*. Other problems with silvicide use included mortality of adjacent nontarget trees.

Roguing (uprooting and removal of oak trees) as a barrier by itself did not prove effective in early treatments because the root system was not broken up sufficiently to halt the spread of *C. fagacearum*. It is currently recommended only in conjunction with trenching. The results of trenching and roguing are discussed later.

MATERIALS AND METHODS

Data analyzed in this review came from post suppression evaluations of 236 oak wilt centers that were trenched as part of the Project. A post suppression evaluation (PSE) is an appraisal conducted by the oak wilt staff at periodic intervals after treatment to determine the effectiveness of a given treatment method for halting the tree-to-tree spread of expanding oak wilt centers. Every treated center is evaluated in the spring and fall for five years following treatment to determine how well each treatment is containing the disease. During a PSE, the entire length of the trench is inspected for breakouts. A breakout is defined as an oak wilt infection in the live oaks immediately beyond the trench. A breakout seldom means the whole trench has failed. It only means that at one point along the trench, the pathogen infected trees just outside the trench.

After an oak wilt center has been positively diagnosed, a treatment plan is custom tailored to fit the site and to meet the objectives of the Project and the landowner. If the disease is in the live oak population, trenching is almost always recommended to sever any roots that are shared between healthy and diseased trees. The proposed trench is located roughly 100 ft (75 ft in early treatments as explained later) from the last symptomatic live oak. The exact placement varies depending on the site (slope, utilities, buildings, etc.).

The recommended trench depth is three ft, but again this varies depending on the site and the type of equipment used. A rocksaw is used on urban sites that are rocky. A dozer with a three-ft-long ripper bar is usually used on rural sites with few obstacles. Other types of equipment, such as chain trenchers and backhoes, can and have been used on other sites. The piece of equipment used depends on the soil depth, the disturbance made to the landscape, the

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terrain, availability, or whatever makes one machine better than another for that particular site.

Treatments can vary (within the guidelines) from one site to the next because no two sites are the same. Differences in treatments vary depending on what the landowner can afford; some landowners cannot afford to save their trees even with the help of the Project. Other landowners can afford to do everything possible. Most landowners can attempt some control measures with the help of the Project.

RESULTS AND DISCUSSION

Of the 236 treated centers that were evaluated, 48 had breakouts. At the time of the last PSE, approximately 80% of the treated centers were holding without any breakouts. It is not a valid analysis to compare all of the treated centers to each other. Although all of the centers were trenched, the trenches were installed in different years, at different times of the year, and with different types of equipment.

This review compares frequency of breakouts in treated oak wilt centers by:

- calendar year of trench installation
- time after installation to breakout
- breakouts per 1,000 feet of trench
- month of treatment installation
- land use category
- type of trenching equipment

The Texas Oak Wilt Suppression Project began in 1988. Fifteen centers were treated during that calendar year. The number of treated centers increased to 43 in 1989, 50 in 1990, and 128 in 1991.

As shown in Figure 1, 80% of the trenches installed in 1988, 42% in 1989, and 18% in 1990 have had at least one breakout. Only 8% of the centers treated in 1991 have had a breakout. This trend suggests either that; 1) the longer a trench has been installed the more likely it is to have a breakout, or 2) more trenches are holding because Project personnel have gained experience in correctly placing containment trenches. The following comparisons suggest that the latter is the most likely explanation for observed differences between years.

The percent of treated centers still holding without a breakout at six-month intervals (Table 1) shows that most breakouts occur within the first twelve months after installation. In 1988, only 60% of the treatments were still holding after six months, and 27% after twelve months. After eighteen months, the number of 1988 trenches holding appears to have stabilized at 20%. In 1990, 98% were holding after six months, 88% after twelve months, and 80% after 18 months. This would tend to indicate that Project personnel are getting better at installing containment trenches that hold beyond the first twelve months.

Based on the results of the treatments that were installed in 1988 and 1989, the guidelines for trench placement were changed beginning in 1990 from 75 ft to 100 ft beyond the last symptomatic live oak. The Project's Technical Advisory Board had initially recommended 75 ft based on aerial infrared photography that showed that oak wilt centers spread at about 75 ft per yr in Texas (Appel and Maggio 1984). The trench is placed approximately one yr ahead of the advancing disease front.

The high number of breakouts within the first 12 mo after trench installation indicated misplacement of the trench. The oak wilt fungus was suspected to be in the asymptomatic live oaks on the outside of the trench before the trench was installed. For this reason the guidelines were changed to read: "construct a trench located at least one hundred (100) feet from the outermost symptomatic trees of the oak wilt center. When the nearest asymptomatic tree is greater than 100 ft from the last symptomatic tree, the trench shall be located so that a buffer tree is between the last symptomatic tree and the trench."

The results of this change are evident in the percentage of trenches installed in 1990 and 1991 that did not have a breakout after 12 mo (88% and 93%, respectively) compared to the percentage of trenches installed in 1988 that did not have a breakout after 12 mo (27%). Every year Project personnel have installed trenches that appear to be holding better than the year before.

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Figure 1. Percent of treated centers with at least one breakout by the calendar year in which the trench was installed as part of the Texas Oak Wilt Suppression Project from 1988 to 1991.

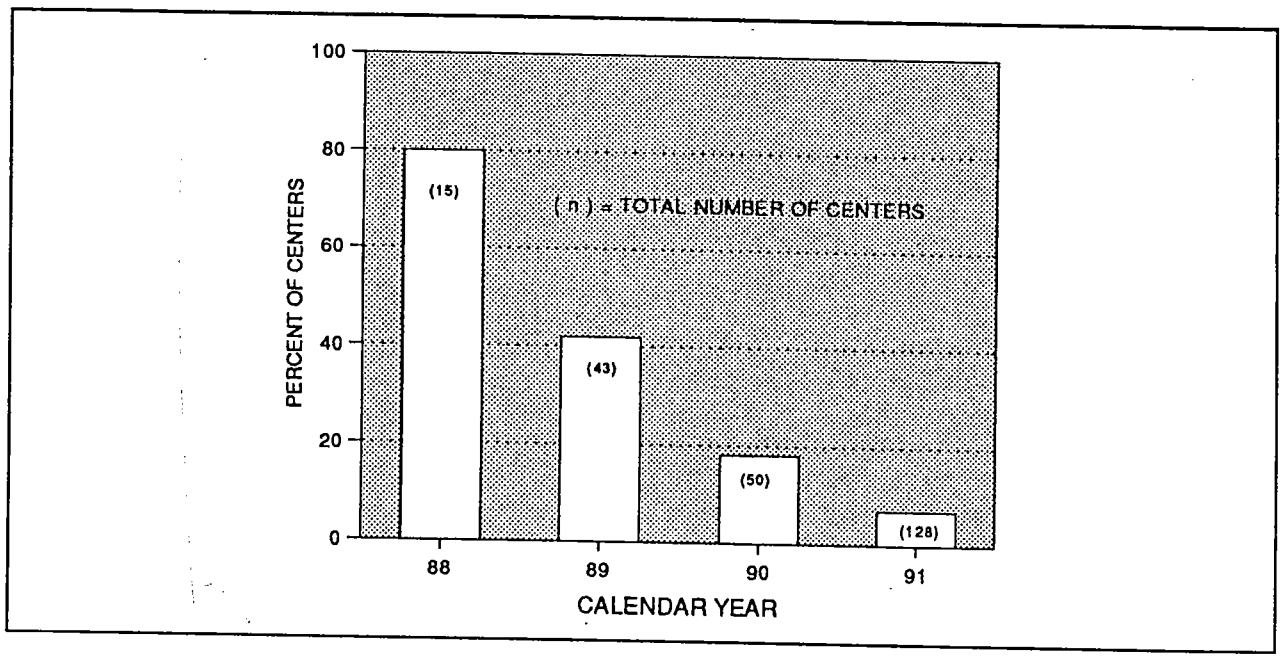

Table 1. The percent of treated centers without a breakout at six month intervals after trench installation (Texas Oak Wilt Suppression Project).

Calendar Year Treated	Total Centers Treated[1]	% of Treated Centers Without a Breakout at: 6 Months	12 Months	18 Months	24 Months	30 Months
1988	15	60	27	20	20	20
1989	43	93	74	65	80	NDA[2]
1990	50	98	88	80	NDA	NDA
1991	128	99	93	NDA	NDA	NDA

[1] Excludes centers that lack PSEs or were not trenched.
[2] No data available due to insufficient time lapse since treatment.

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Figure 2. The percent of treated centers with breakouts by month of treatment installation as part of the Texas Oak Wilt Suppression Project (1988-1991).

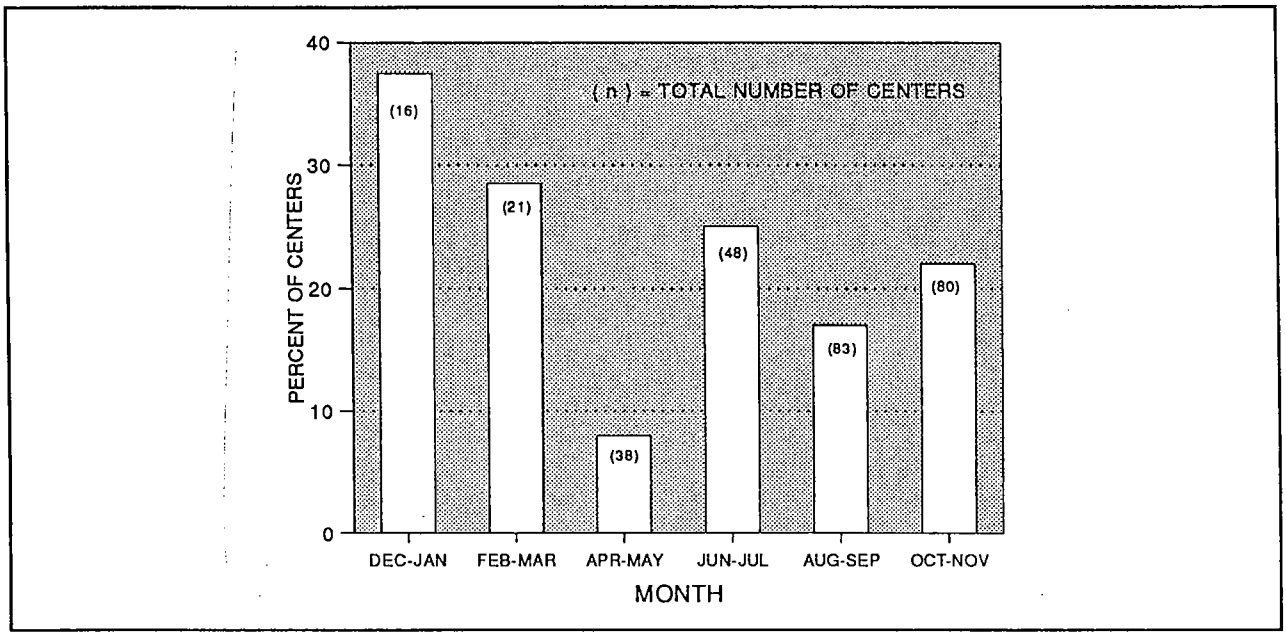

Table 2. The number of breakouts per 1,000 feet of trench at six month intervals after trench installation (Texas Oak Wilt Suppression Project).

Calendar Year Treated	Total Breakouts[1] (Centers Treated)	# of Breakouts per 1000 ft of Trench at: 6 Months	12 Months	18 Months	24 Months	30 Months
1988	16(15)	0.288	0.672	0.769	0.769	0.769
1989	23(43)	0.073	0.203	0.332	0.424	ND A[2]
1990	12(50)	0.023	0.094	0.141	NDA	NDA
1991	8(128)	0.008	0.031	NDA	NDA	NDA

[1] Includes multiple breakouts per center.
[2] No data available due to insufficient time lapse since treatment.

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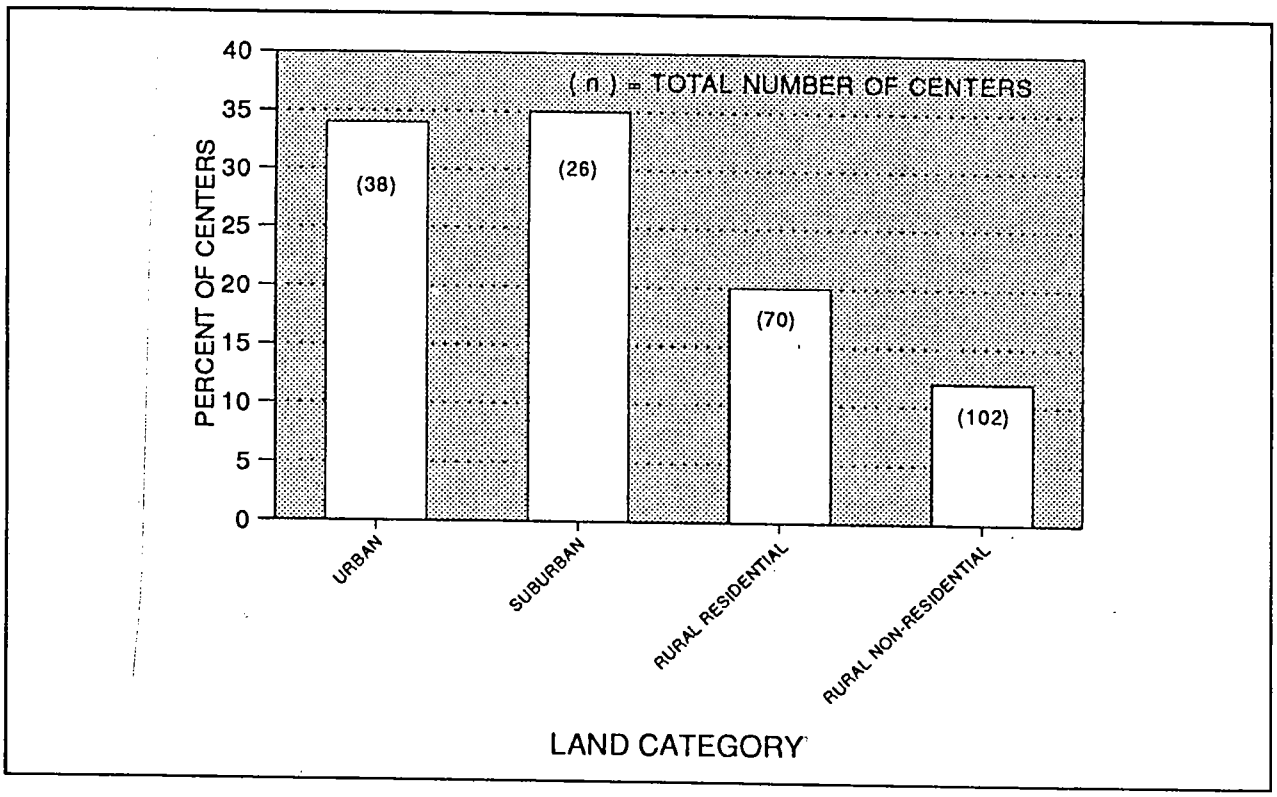
Figure 3. The percent of trenched centers with breakouts by land use as classified in the economic analysis by McKinney and Billings in these proceedings (Texas Oak Wilt Suppression Project 1988 - 1991).



In a separate analysis, a comparison was made of the number of breakouts per 1,000 ft of trench at six- mo intervals after trench installation for each year (Table 2).

In 1988, after 12 mo, there was approximately 1 breakout for every 1,500 ft of trench that was installed. For trenches installed in 1991, after 12 mo there was approximately one breakout every 32,000 ft. Two facts contribute to the differences in these numbers. First, fewer breakouts occurred within the first 12 mo on treated centers installed in 1991 compared to 1988. Second, considerably more centers were treated in 1991 than in 1988. The improvement from 1988 to 1991 was from almost one breakout within the first .year per center treated to one breakout within the first year for every 14 centers treated. (The average center contains about 1,700 ft of trench.)

The comparison of the percent of centers with breakouts by month of treatment (Figure 2) shows a surprising seasonal difference. Overall, about 20% of the centers had a breakout. Treatments installed from December to March had a higher breakout rate than the overall average. Those installed from June to November were approximately the same as the overall average. For reasons that remain unclear, only about 8% of the treatments installed in April and May had a breakout.

One possible explanation for the better results in April and May could be that symptom development most accurately corresponds to disease progression at this time of year. Perhaps future research will

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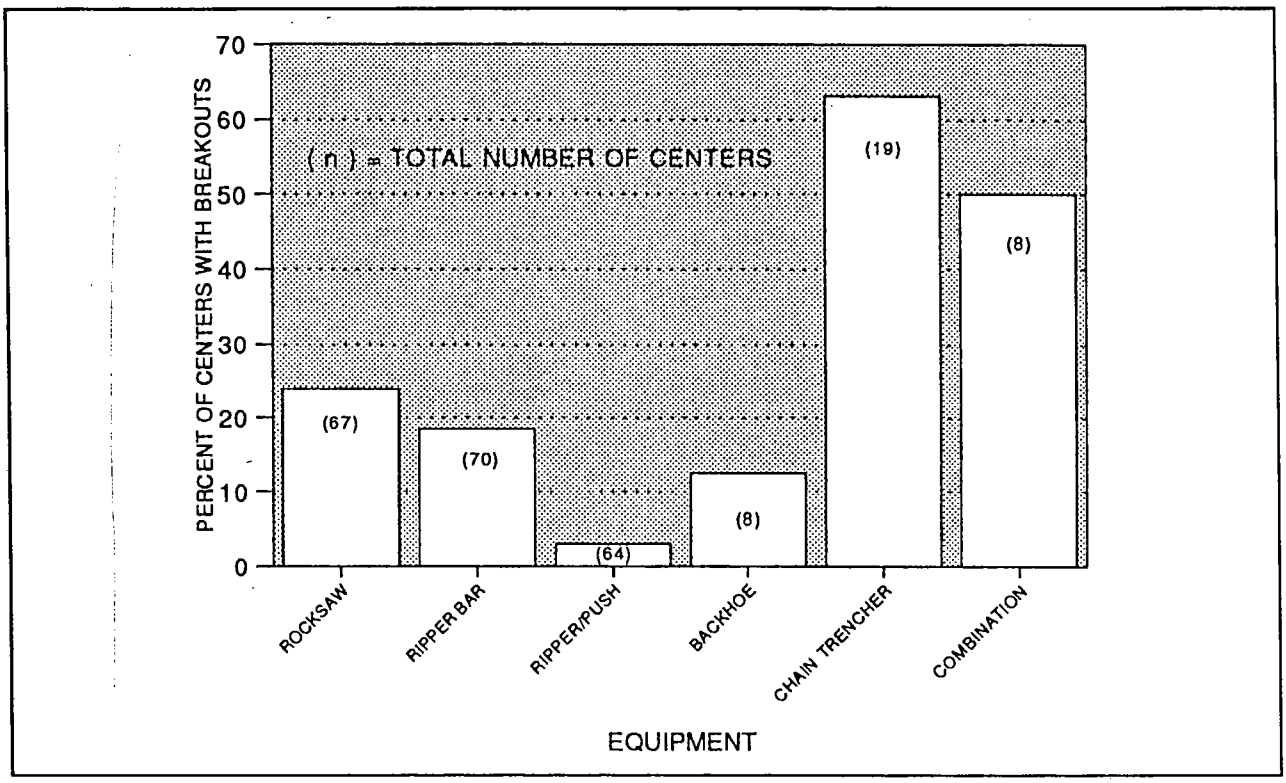
Figure 4. The percent of treated centers with breakouts by the type of equipment used to install the trench (Texas Oak Wilt Suppression Project 1988 - 1991).



explain this trend. Or, as the Project continues, seasonal differences could possibly change.

Figure 3 illustrates the percent of treated centers with a breakout by land use. For this analysis all the treated centers were placed into one of four land use categories: urban, suburban, rural residential, or rural non-residential (see McKinney and Billings, these proceedings). Results suggest that trenches in rural non-residential or rural residential areas suffer fewer breakouts than in urban or suburban areas. Urban areas are more difficult to trench because of houses, roads, utilities, and other obstacles not found in rural areas. In an urban environment, dead trees are more likely to be promptly removed and there are more stresses that can cause a tree to look unhealthy. The resulting stumps and stressed trees obscure disease progression, making correct trench placement more difficult.

Unlike urban centers, rural centers are usually confined to one or two landowners. Without the need to obtain the cooperation of several landowners, the time between diagnosis and treatment of rural centers is considerably less than for urban centers. Another reason why results in rural sites tend to be better than urban sites may be because it is easier to convince landowners to sacrifice trees in a rural setting to save the remaining trees. Removal of asymptomatic oaks within the trench may reduce incidence of breakouts by limiting disease spread.

The percent of centers with breakouts by equipment type (Figure 4) generally reflects the comparison by land use. The two pieces of trenching

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equipment used most often were rocksaws or bulldozers (D8 with 3-ft ripper bar). The rocksaw was used primarily in urban areas or around houses in rural areas. The dozer was used to install trenches in rural areas with few obstacles. The best results were obtained by installing a trench with a ripper bar and pushing (roguing) the live oaks within the trench line. Roguing by itself did not adequately breakup the root system, but when combined with ripping provided very good results.

The least effective piece of equipment for oak wilt containment appeared to be the chain-type trencher. It is important to note that chain trenchers were used on the first few centers that were treated. These centers had breakouts probably due to the trench being misplaced. Also, the chain trenchers had trouble cutting through the shallow, rocky soils common to central Texas. Combinations usually involved a chain-type trencher and a rocksaw. The chain trencher would go deeper in deep soils, while the rocksaw would cut through rocky areas. A backhoe is an alternative to using combinations because it can dig deeper and go through loose rock.

SUMMARY

In the first four years of operation, the Texas Oak Wilt Suppression Project has been successful. The Project, however, has not been without problems. It has evolved to overcome many of these problems. Some technical recommendations made at the initiation of the project have been dropped and others modified, resulting in increased effectiveness of direct control treatments to suppress oak wilt in central Texas.

The direct control treatment that has become the cornerstone of the Project is trenching. Trenching is not always totally effective, but the technical guidelines are continually being modified to increase the success rate. One of the major changes has been in trench placement (increasing the distance from the last symptomatic live oak to the proposed trench to at least 100 ft). Other changes involved the use of existing barriers, more backup and secondary trenches, and the elimination of silvicide barriers.

Many of the breakouts that were observed occurred where streets, existing utilities, or natural barriers (creeks, cliffs, etc.) were used as barriers. These existing barriers are no longer used unless there is a history of disease progression along one side of the barrier and not the other. Where streets or other obstacles that cannot be crossed are encountered, trenches in the form of "T"s or "L"s are cut parallel to the obstacle to sever any roots that might be crossing at an angle.

Backup and secondary trenches are installed in areas that are likely to break out, based on past experience. A backup trench is a trench located beyond the primary trench and a secondary trench is a trench located between the symptomatic trees and the primary trench. An area considered for additional trenching is where the disease appears to be advancing at a rate different than the norm (either faster or slower). A backup trench also might be used when, according to the guidelines, the trench should go through a house, drain field, or other obstacle. Installing a trench closer than the guidelines specify might halt the disease, but a backup trench would be in place if it did not.

Disease suppression is both an art and a science. The key to successful suppression is a blend of the two. If the trends in this review are an indication of successful disease management, then the Texas Oak Wilt Suppression Project is approaching an optimal blend.

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Economic Analysis of Oak Wilt Suppression in Texas

Chuck McKinney
Ronald F. Billings

A benefit:cost analysis of treatments installed in the first five years of the Texas Oak Wilt Suppression Project was conducted. The estimated value of trees saved from oak wilt, caused by the fungus *Ceratocystis fagacearum*, due to installation of direct control treatments was compared to the cost of treatment installment as well as the total cost of the Project. Results indicate that control treatments of trenching and/or fungicide injections are economically justified. Also, the Suppression Project is cost effective. For every $1.00 invested in the project, an average of $2.60 is accrued in value of trees protected, based on a single year of protection from oak wilt. Benefits were greatest on residential properties, due to the high value of live oaks as shade trees in central Texas.

INTRODUCTION

In 1988, a cooperative project was initiated within the State of Texas to minimize the spread of oak wilt, a disease of oak trees caused by *Ceratocystis fagacearum* (Bretz) Hunt. The Texas Oak Wilt Suppression Project is administered by the Texas Forest Service, with cooperation from the USDA Forest Service, the Texas Agricultural Experiment Station, the Texas Agricultural Extension Service, the Soil Conservation Service, and the City of Austin (see Cameron and Billings, these proceedings).

Specific objectives of the Texas Oak Wilt Suppression Project include the following:

1. Initiate or accelerate public awareness of oak wilt and educate urban and rural landowners in central Texas of the oak wilt threat, including prevention and suppression alternatives.
2. Identify current oak wilt centers in selected suppression areas.
3. Provide ground verification and diagnosis of oak wilt infection centers located from detection surveys and other sources.
4. Notify landowners of oak wilt centers and provide control recommendations; assist with implementation of control treatments, including sharing of control costs.
5. Conduct post suppression evaluations of direct control treatments to determine effectiveness in stopping the spread of oak wilt (see Gehring, these proceedings).
6. Establish a computerized record-keeping system of detection, ground evaluation, and control information.

OBJECTIVE

The purpose of this economic analysis was to address the question "Was the money invested in the Texas Oak Wilt Suppression Project since June, 1988, well spent?" To answer this question, the value of trees saved from oak wilt infection as a direct result of Project activities (benefit) must be determined and compared to the total funds spent on the Project (cost). A benefit:cost ratio greater than one would imply a cost-effective investment. For purposes of this economic analysis, benefits were limited to the value of trees protected by trenching, tree removal, and/or fungicide injection. The analysis excludes additional benefits that may accrue from other Project activities such as public education, prevention, infected red oak removal, and herbicide treatments.

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COMPONENTS OF THE ECONOMIC ANALYSIS

Project Costs

Federal funds (50%) allocated annually for the Texas Oak Wilt Suppression Project were $169,000, $345,000, $345,000, $465,000, and $450,000 from 1988 to 1992, respectively. This represents a five-year total of $1,775,000. Actual expenditures of federal funds through August 31, 1992, however, totalled $1,609,000. These funds, in turn, were matched by funds from the Texas Forest Service (32%), the City of Austin (3%), and participating private landowners (15%).

As described in a previous paper (Cameron and Billings, these proceedings), qualified landowners who follow an approved oak wilt treatment plan are reimbursed with federal funds for one half the costs of implementing control treatments. Cost-sharable treatments consist of; 1) trenching around oak wilt centers, 2) tree felling and removal within trench boundaries, 3) tree pushing within trench boundaries, 4) infected red oak removal, 5) herbicide applications (prior to 1991), and 6) fungicide injection for high value oak trees within trench boundaries.

From June 1, 1988, to September 1, 1992, a total of $848,740 was spent on cost-shared oak wilt treatments. Of this total, 66.6% was spent on trenching, 14.6% on tree felling and removal, 4.5% on tree pushing, 4.5% on infected red oak removal, 0.1% on herbicide treatments, and 9.7% on fungicide injections with the fungicide Alamo® (propiconazole).

Land-Use Classification

To facilitate the economic analysis, all oak wilt centers treated with Project funds prior to September 1, 1992 were classified into one of four broad land-use categories (Anderson et al. 1976). These categories were: (1) urban residential, (2) suburban residential (1-10 ac homesites in a transition area between urban and rural), (3) rural residential (homesite on farm or ranch land of more than 10 ac), and (4) rural non-residential (crop or range land).

Host Densities and Tree Values

To characterize the tree cover on each of the four land-use categories mentioned above, six treated oak wilt centers were randomly selected from aerial photographs in each land-use category. Each center was visited by field crews and the tree cover surveyed systematically by means of 1/5 or 1/10 ac plots. Survey plots were established at two-chain (132 ft) intervals parallel to and outside the trench line of each center. Measurements recorded for each tree within the survey plots consisted of tree species, tree diameter, and numerical values for trunk condition, growth, structure, presence of insects or disease, crown development, life expectancy, and location on the property (proximity to dwellings).

Using the International Society of Arboriculture (ISA) formula described by Dreeson (1988), percentage values of tree class, condition, and location were estimated for each tree within the survey plots. The value of individual trees was computed with the formula:

$$\text{Tree value} = 0.7854(\text{diameter}^2) \times \$50 \times \text{class percentage} \times \text{condition percentage} \times \text{location percentage}.$$

Results of these appraisals on a per tree and per acre basis for each of four land-use categories are shown in Table 1.

Although the ISA formula provides a reasonable estimate of central Texas tree values in urban areas, we believe it grossly overestimates tree values for the other land-use categories. This is particularly true for rural non-residential areas where total land value (with or without trees) seldom exceeds $4,000/ac. Accordingly, an alternative method was chosen to estimate tree values for this study. Values for trees per acre in each land-use category were estimated as a percentage of the average land value (trees and houses included). Martin (1986), in assessing the impact of oak wilt in Austin, TX, reported that live oaks and red oaks contributed 19% of the total property value.

For purposes of our economic analysis, we used average real estate values of $300,000/ac for urban, $150,000/ac for suburban, $50,000/ac.

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Table 1. Estimated values per tree and per acre for live oaks and red oaks in the vicinity of oak wilt centers within four land-use categories, based exclusively on the ISA formula method of appraisal (Dreeson 1988).

Category	Trees/Acre	Ave. DBH (in)	$/Tree	$/Acre
Urban	16.7	16.5	$ 4,186	$79,926
Suburban	72.2	9.8	$ 734	$52,995
Rural residential	20.8	16.4	$ 2,660	$55,328
Rural non-residential	32.6	11.7	$ 600	$19,560

for rural residential, and $1,500/ac for rural non-residential categories. In turn, the percentage of total value due to tree cover was estimated at 20%, 15%, 30%, and 50% of total real estate value for the land-use categories, respectively. This yielded a tree value per acre of $60,000 for urban settings, $22,500 for suburban settings, $15,000 for rural residential settings, and $750 for rural non-residential settings. Average value for an individual tree, based on the trees per acre values shown in Table 1, were computed to be $3,593 (urban), $312 (suburban), $721 (rural residential), and $23 (rural non-residential). These average tree values, considered more realistic in our opinion than the ISA tree values shown in Table 1 (particularly for rural settings), were used in subsequent benefit:cost comparisons.

Rates of Disease Spread

Most transmission of *Ceratocystis fagacearum* is via root grafts or, in the case of live oak (*Q. fusiformis* Small and *Q. virginiana* Mill.), interconnected root systems. Accordingly, most tree losses in live oak stands in central Texas result from tree-to-tree expansion of established oak wilt infection centers (see Appel, these proceedings). Appel et al. (1989) developed a computer-based system for measuring the rate of spread of expanding oak wilt centers in live oak stands in central Texas. Despite considerable variation among centers, these authors estimated an average spread of 75 ft per yr over a 3-5 yr interval. When oak wilt infection centers were subdivided into eight pre-shaped divisions (functional octants, oak wilt centers tended to spread in an average of 6.2 octants in urban and suburban situations and 5.5 octants in rural situations. Accordingly, these rates and characteristics of spread were utilized in the economic analysis to estimate numbers of trees saved with oak wilt trenches per land-use category.

Project Accomplishments

Between June 1, 1988, and September 1, 1992, the Texas Oak Wilt Suppression Project installed 640,666 ft of trench. These efforts were divided among urban residential sites (69,832 ft), suburban residential sites (46,128 ft), rural residential sites (181,308 ft), and rural non-residential sites (343,398 ft). In addition, 1,761 trees were injected with the fungicide Alamo®, (propiconazole), primarily in urban and rural residential areas.

Other Assumptions

Certain assumptions on rates of oak wilt spread and effectiveness of control treatments were made to facilitate this economic analysis. Among these were the following:

1. Without control treatments (trenches), oak wilt centers will expand at 40 ft/yr in urban areas, 60 ft/yr in suburban areas, and 75 ft/yr in rural areas.

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2. Ninety-five percent of live oaks will be killed or seriously damaged by oak wilt as infection centers expand.
3. Although 20% of trenches suffer breakouts (failure to halt the spread of oak wilt), the proportion of the total footage of installed trenches estimated to be successful averages 97.5% (see Gehring, these proceedings).
4. Of oaks injected with the fungicide propiconazole (Alamo®), 50% will be protected from oak wilt infection.

Estimating Trees Saved By Oak Wilt Treatments

To estimate the number of live oaks saved from oak wilt infection as a direct result of trenching around expanding infection centers, the total feet of trench installed was multiplied by the average annual spread of oak wilt for each land-use category (Table 2). This product was then multiplied by the proportion of functional octants to total octants (to account for directional spread), by the estimated percentage of oaks affected (95%), and by the average percentage of trench line not affected by breakouts. The final area was converted to acres to derive the average acreage protected for one year in each land-use category.

The dollar value of trees protected per year was computed as the product of estimated acres affected per yr and tree value per ac for each land-use category (see Table 2 for details). Based on these computations, average value of trees protected by halting oak wilt spread for a single year with project-funded trenches totalled an estimated $6,985,050. Values saved were highest on rural residential sites ($2,982,000) and lowest on rural non-residential sites ($285,300).

The additional value of trees saved by fungicide (Alamo®) injection was estimated as the product of total trees injected, survival at the end of a two-yr period following injection (estimated at 50%), and average value per tree within each land-use category. The value of trees surviving oak wilt following fungicide injection was estimated to total $1,486,220 for all land use-categories combined. Most of this total value (81%) was attributed to saving high value, large-diameter trees in urban residential settings (Table 2).

BENEFIT:COST RATIOS

Based on computerized records of the Texas Oak Wilt Suppression Project, combined with the above-mentioned assumptions, benefit:cost ratios for each of four land-use categories were computed for oak wilt treatments, as detailed in Table 2.

Based solely on direct costs of installation, treatments involving trenching and tree removal to prevent continued spread of oak wilt appear to be cost effective. The average benefit:cost ratio for all land-use categories combined was 9.6:1 (Table 2). The benefit:cost ratio was greatest for rural residential areas (13.1:1), and least for rural non-residential areas (2.0:1). In urban areas, the high value of individual trees (estimated to average $3,593) is offset by the high cost to install a trench and remove trees ($4.05/ft). In contrast, trenches and tree removal are inexpensive in rural, non-residential areas ($0.41/ft), but individual trees average very low in value ($23/tree).

When the costs and benefits of Alamo® treatments are factored in, the average benefit:cost ratio over all land-use categories becomes 8.5:1 (range 1.7-10.6:1). Interestingly, data in Table 2 suggest that the costs for injecting trees with Alamo® in rural non-residential areas exceeds the benefits by approximately 12 to 1, due to the low average value of individual trees. Presumably, however, the 211 trees targeted for fungicide injection in rural non-residential areas were those considered of particularly high value by landowners who contributed one-half the cost of injection.

To ascertain whether the Texas Oak Wilt Suppression Project is cost-effective as a whole, total project benefits of trenching, tree removal, and fungicide injections ($8,467,261) were divided by total project expenditures prior to September 1, 1992 ($3,218,000). The result is a benefit:cost ratio of 2.6:1. This is still a respectable outcome. It should be emphasized that benefits in this evaluation are based on stopping oak wilt infection spread for a single year. In reality, oak wilt infection centers may expand at least five yr in the absence of control

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(Appel et al. 1989). Thus, project benefits will double at no additional costs for each succeeding year that trenches and fungicides are effective following date of application. Assuming treatments remain effective for five yr, the benefit:cost ratio for 1988-1992 treatments based on total project expenditures would eventually be an estimated 13.0:1. Furthermore, these benefits do not include trees saved from other Project-funded activities such as prevention of new oak wilt centers through public education and infected red oak removal.

In summary, using conservative estimates of benefits, we conclude that the Texas Oak Wilt Suppression Project in general, and recommended control treatments of trenching, tree removal and fungicide injection in particular, are cost effective approaches to reducing losses to oak wilt in central Texas. This is especially true in situations where high value live oaks are threatened by expanding oak wilt centers in proximity to residences in rural, suburban, and urban settings. Benefits will continue to accrue as long as treatments are effective in preventing the spread of oak wilt. Only time will tell how long installed oak wilt control trenches and fungicide injections will remain effective for protecting oak trees in central Texas.

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Table 2: Texas Oak Wilt Suppression Project economic analysis, based on tree value as an percentage of total real estate value.[1]

	Factor	Land Classification				
		Residential Urban	Residential Suburban	Rural Residential	Rural Non-Residential	Total
A.	Ft of trench	69,832	46,128	181,308	343,398	640,666
B.	Cost/ft (trench only)	$3.19	$1.34	$1.07	$0.29	$0.89
C.	Cost/ft (trench+removal)	$4.05	$1.70	$1.26	$0.41	$1.14
D.	Total trench cost (AxC)	$282,755	$78,418	$228,448	$140,793	$730,414
E.	Average annual spread (ft)	40	60	75	75	
F.	Functional octants	6.2/8	6.2/8	5.5/8	5.5/8	
G.	% Oaks affected	95%	95%	95%	95%	
H.	% with breakout (holding)	34% (0.957)	35% (0.956)	20% (0.975)	12% (0.985)	20%
I.	Average value/ac (with houses)	$300,000	$150,000	$50,000	$1,500	
J.	Tree value as % of property value (I)	20%	15%	30%	50%	
K.	Tree value/acre (IxJ)	$60,000	$22,500	$15,000	$750	
L.	Acres protected/yr [AxExFxGx(H)]/43,560	45.2	44.7	198.8	380.4	669.1
M.	Average value protected (KxL)	$2,712,000	$1,005,750	$2,982,000	$285,300	$6,985,050
N.	Benefit:cost for trench (M/D)	9.6:1	12.8:1	13.1:1	2.0	9.6
O.	Average dbh/tree	16.5	9.8	16.4	11.7	13.6
P.	Average trees/ac	16.7	72.2	20.8	32.6	35.6
Q.	Average value/tree (K/P)	$3,593	$312	$721	$23	$1,162
R.	Trees injected with Alamo	669	194	687	211	1,761
S.	Estimated % survival of oaks following Alamo injection (actual value to be determined)	50%	50%	50%	50%	50%
T.	Average dbh of injected tree	16	10	16	14	14.9
U.	Value of surviving trees [RxSxQ]	$1,201,858	$30,264	$247,663	$2,426	$1,482,211
V.	Costs of injection ($10/dia. in)	$107,040	$19,400	$109,920	$29,540	$265,900
W.	Benefit:cost for Alamo (U/V)	11.2:1	1.6:1	2.3:1	0.1	5.6:1
X.	Total estimated benefit (M+U)	$3,913,858	$1,036,014	$3,229,663	$287,726	$8,467,261
Y.	Total estimated costs (D+V)	$392,795	$97,818	$338,368	$170,333	$999,314
Z.	% for Alamo (V/Y)	27%	20%	32%	17%	27%
AA.	Benefit:cost (trench+Alamo) (X/Y)	10:1	10.6:1	9.5:1	1.7:1	8.5:1

Estimated Project benefit:cost (trenches+tree removal)=$6,985,050/$3,218,000=2.2:1
Estimated Project benefit:cost (with Alamo) $8,467,261/$3,218,000=2.6:1

[1] Data incorporated in this economic analysis cover the interval from June 1, 1988 to September 1, 1992 and represent a more complete and comprehensive evaluation than that presented at the National Oak Wilt Symposium.

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The Role of Extension in Oak Wilt Management

Jerral D. Johnson

Extension personnel through meetings, publications, and various forms of mass media have been actively involved with the oak wilt program in Texas. The Extension staff in many counties in which oak wilt has been identified report 20 or more telephone calls a day in regard to oak wilt and its control. This plant disease affects more people than any other single disease in Texas. Homeowners and others that own property in or near oak wilt centers have experienced substantial losses in land values. The loss of browse has affected wildlife in rural areas where oak wilt is present. Oak acorns are a major source of food for wildlife in the Texas Hill Country. This loss of food translates into less wild game. Less game can reduce the amount received for hunting leases and the demand for those leases. Extension has the responsibility of educating and training individuals in the identification and control of this major plant disease. The Plant Disease Diagnostic Laboratory is operated by Extension to aid in the diagnosis of this problem.

INTRODUCTION

Extension plant pathology specialists have played a role in the development of an oak wilt management program. Extension pathologists, county Extension agents, county horticulturists, and other professionals involved with trees work together to create an awareness of this major oak disease and to assist clientele in disease management. A program similar to the "Master Gardener" program was established in Hays county to provide support to property owners that had or suspected the presence of oak wilt on their property. This program was operated from the County Extension Agent's office. This proved to be an excellent method of reaching a larger number of individuals within the county. Oak wilt affects a large percentage of the land and population in Texas. The current and potential impact of this fungus on Texas clientele is outlined in Table 1.

EIGHT STEP PROGRAM FOR OAK WILT MANAGEMENT

Extension plant pathologists, working jointly with Texas Agricultural Experiment Station research plant pathologists, members of the Texas Forest Service, and agribusiness representatives, developed an eight point management program. The program is directed at controlling active oak wilt centers and also to property owners who are not currently in an oak wilt center but want to prevent exposing their trees to the fungus.

First Step: Identify the Cause of the Tree Loss

Identification and understanding the disease is an important step in any management program. The disease is identified by foliage symptoms, rate of tree mortality, rate of spread of the fungus, and the type of trees infected by the fungus. The Texas Plant

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Table 1. Impact of oak wilt on Texas property and clientele.

Total Land Mass	**% Infected**	**Accumulated**
A. Counties with confirmed oak wilt	18	---
B. Counties at risk (1-2 counties adjacent to counties in A)	36	54
Population	**Percent**	
A. Counties with confirmed oak wilt	43	---
B. Counties at risk (1-2 counties adjacent to counties in A)	31	74
C. Counties within 3-5 counties with oak wilt	16	90
Metropolitan Centers in Oak Wilt Counties	**Percent**	
A. Counties with confirmed oak wilt	32	---
B. At risk (1-2 counties adjacent to counties in A)	18	50
C. Counties within 3-5 counties with oak wilt	29	79

Disease Diagnostic Laboratory at College Station makes tissue isolations from suspect trees to confirm the oak wilt fungus.

Second Step:
Isolate the Diseased Trees With Trenches

Once the disease has been identified in an area, diseased trees are isolated from surrounding healthy trees. Trenching is the most effective method. Trenches are established at least 100 ft in front of the last symptomatic tree. Trenches are 36-40 in. deep.

Third Step: Tree Removal

Once the trench is established, trees with more than 30% of their canopy showing wilt symptoms are removed. All diseased Spanish oaks (*Quercus texana* Buckl.) and blackjack oaks (*Q. marilandica* Muenchh.) are removed as soon as they develop symptoms. Immediate removal of these trees reduces the possibility of insect spread.

Fourth Step: Pruning

Pruning cuts and wounds are areas where infection occurs. Sap droplets formed on the surface of the cuts or wounds are fed on by nitidulid beetles. It is suggested that spores carried on the bodies of the insects or in fecal material is deposited on the cut surface. New infections result from this spread. To prevent this infection, do not prune between February 1 and June 15 or October 15 and December 15. These are times when beetles and fungal mats are active.

Apply wound paint to fresh-cut or damaged surfaces to protect from oak wilt infection. Wound paints act as a barrier to fungus-carrying beetles. Apply the paint immediately after making the cut. To be cautious always use wound paint when working on trees within one mi or less of an oak wilt center. At distances greater than that, apply the wound paint during months of insect and mat activity. Although the sap-feeding beetles may not fly more than a mile, they can be caught in spring storms and blown for greater distances. In addition there may be diseased

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firewood in the area and this could be a source of the fungus-carrying sap-feeding beetles. Wound paint is not suggested for trees other than those that are susceptible to oak wilt.

Fifth Step: Firewood Control

Firewood from diseased Spanish and black jack oaks is another method of spreading the fungus. Nitidulid beetles visit diseased logs on which fungal mats are formed and then spread spores to nearby healthy trees. It is suggested that firewood from an unknown source be covered with clear plastic to prevent insect spread. The oak wilt fungus in wood that has been cut and stored in the sun for several months will no longer be active. Wood from live oak trees is not considered to be a carrier for the fungus, since mats have never been observed on live oaks.

Sixth Step: Alamo® Injections

Trees on the diseased side of the trench that have 30% or less of their canopy with wilt symptoms or trees that are infected but do not yet have wilt symptoms may be injected with the systemic fungicide Alamo®. This treatment has proven to be an effective treatment for saving individual trees. However, it will not prevent the spread of the fungus. Chemical injections should be used in conjunction with the other control steps. Injections are not suggested for trees that are more than 500 ft from a diseased tree. Although the pesticide is labeled for two and three ml per in. of trunk diameter, it is suggested that it be used only at the 3 ml rate. Large trees that are immediately adjacent to a diseased tree should be retreated 12 mo after the first injection. Make this second injection at the base of the tree and not on the root flares.

Seventh Step: Monitor

It is important to observe trees for new symptom expression following treatments for oak wilt. Trees should be monitored for disease development and steps taken immediately to prevent additional spread.

Eighth Step: Replanting

Data from replanting studies indicate that Spanish oaks and live oaks planted in an oak wilt center are not likely to be infected by the fungus. Protect young trees from insect infection. Root grafts are not likely to occur for several years after a tree has been planted.

To reduce the risk of tree loss in the future, property owners are encouraged to plant a mixture of trees and not depend on a single species. Increased use of bur oaks in the planting has been suggested. Chinese pistachio, bald cypress, several species of elms, flowering pears (Bradford and Aristocrat) and sycamore (in deep well drained soils along creek and river beds) are non-oak species that are recommended for planting.

EXTENSION'S ROLE IN OAK WILT EDUCATION

Create an Awareness of Oak Wilt

The Extension Service has a role to play in all three phases of the oak wilt program. It has the primary responsibility for creating an awareness of the problem. There have been many theories as to what is killing oak trees in Texas. Extension specialists and agents serve as a source of information for individuals on the cause of the problem and what can be done. The Extension Service is well equipped to handle this phase of the program. Agents are located in all counties where oak wilt is a problem. There are nine Extension specialists located in different areas of the state to serve as support for agents and to work directly with property owners. A Plant Disease Diagnostic Laboratory is maintained on the Texas A&M University campus at College Station to diagnose tree problems. The lab currently handles over 600 tree specimens each year. Many of these are suspected of having oak wilt.

Applied Research

Members of the Extension Service play a role in the area of applied research. This work is in support of the main research effort conducted by Dr. David

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Appel. Extension Plant Pathology Specialists conduct field trials to demonstrate proven control practices and evaluate new methods. They are supported by County Extension Staff, who can provide day to day observations if required. In addition, the major metropolitan centers have County Extension Horticulturists who provide additional expertise in tree management.

EXAMPLES OF EXTENSION PROGRAMS

Community Control: LaGrange

In the early 1980s, a series of tornados occurred in an area just south of La Grange, known as Monument Hill. Soon after this, trees were observed with oak wilt symptoms. The County Extension Agent requested assistance from the Extension plant pathologist located at College Station. A plan was developed to use a series of trenches to stop movement of the fungus followed by tree removal. The County Extension Agent met with property owners and outlined the plan. All property owners agreed to the plan and each shared in the expense of trenching and tree removal. The program was effective--tree loss was stopped.

Community Control: Cuero

In 1986, oak wilt was identified as the cause of massive oak losses north of Cuero in DeWitt County. Property owners were notified by the County Extension Agent that the problem existed and that they should install a trench to stop the movement and then remove the diseased trees. Only one property owner was interested in the program and trenched his property lines. His trees have remained healthy. All susceptible trees around his property have now died from the disease.

Community Control: Kerr/Bandera Counties

In Kerrville and Gillespie counties, a property owner association representing Terra Linda development asked for help in developing an oak wilt control program for their development. Selected members of the association were given intensive training. They then worked with their neighbors to develop a comprehensive control program for their development. A total of 3,000 trees were injected along with several miles of trenches. In this case, oak wilt has brought a community together to fight a common problem.

Educational Programs: County and Regional Meetings

The primary focus has been the training of individuals in identification and control of oak wilt. Meetings have been held in all counties with oak wilt. Both formal and informal meetings have been conducted to train individuals in the control of this disease. In addition to those conducted by Extension Plant Pathologists, county agents have conducted meetings in their county and also in adjoining counties. Arborists have often been used as resource personnel to conduct the meetings.

Educational Programs: Hays County "Stamp Out Oak Wilt"

Hays County Extension Agent Mr. Cliff Caskey and his county horticulture committee felt that oak wilt was enough of a problem in their county that they needed to make a special effort to stop the loss of this valuable resource. A committee of arborists, homeowners, nurserymen, and county officials was formed to look at the problem and to develop a control program. Extension and research plant pathologists from Texas A&M University, along with members of the Texas Forest Service, worked with the committee to develop a county-wide program.

A program known as "Stamp Out Oak Wilt in Hays County" was started by this committee. Paraprofessionals were trained by the Extension Plant Pathologist to support the county agent. Prepared fliers were placed in windows of stores around the county to publicize the program and to identify individuals in the area that could assist homeowners in the control of this program. To aid in getting information out to the public, one of the major utilities agreed to place a half-page flyer in each month's billing. A total of 12 informational 'bullets'

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were prepared and mailed out. Each 'bullet' targeted a specific part of the program.

The Hays County model has been duplicated in several other counties where paraprofessionals are being trained to assist homeowners with the design and implementation of control efforts. Once trained, the individuals agree to support County Extension Agents in managing oak wilt in their respective counties. They assist the agent in identifying the problem for property owners, work with homeowners in collecting and shipping samples to the laboratory for diagnosis, and provide guidance in tree injection.

Educational Programs:
Tree Health Workshops

In 1991, a series of 'Tree Health' workshops were conducted in Texas. These were one day meetings and were directed to the homeowner and tree professional. The meetings were designed to take advantage of the oak wilt program as a vehicle for developing a complete program in general tree health. Those attending were pleased with the program and requested that similar meetings be held in 1992. The meetings involve speakers from many agencies and private individuals that are involved with trees.

PREPARATION OF INSTRUCTION MATERIALS

Video Tapes and Slide Sets

To reduce travel and to extend the availability of specialists to the public, two video tapes were prepared and made available to interested individuals. The first tape was 90 min in length and discussed the oak wilt management program. This was used by individuals, property owner associations, and others that were interested in the program and in purchasing the fungicide Alamo®. The response was so great that additional tapes had to be made to adequately handle requests. A second 20 min tape was made for use by service clubs, garden clubs, and other groups. This tape was used to create an awareness of the disease and that there was an effective management program.

Two slide sets were prepared and made available for use by County Extension Agents, civic clubs, garden clubs, and property owner associations. The first set is similar to the 90-min video tape. The second set is a detailed description on how to inject trees with the systemic fungicide, Alamo®. Both are available from the Extension office in the Department of Plant Pathology and Microbiology at College Station.

Newsletter

A spin-off of the original oak wilt meetings was the development of a "Tree Health Newsletter." This letter is currently mailed to those individuals that have attended Extension oak wilt meetings or have requested to have their name included on the mailing list. The letter is mailed to 1,500 individuals, three times a year.

Publications

Publications are important in the training of individuals in oak wilt management. The color publication entitled "Major Diseases of Oak Trees" was jointly prepared by Jerral Johnson and David Appel. A second publication, "Strategies For Controlling Oak Wilt," outlines management strategies and was jointly prepared by Wendell Horne and Jerral Johnson. A third publication was prepared as a one-page information sheet on oak wilt management by Jerral Johnson and David Appel. A total of 160,000 copies of these publications have been prepared and handed out to individuals interested in controlling this disease of oak trees.

A POSITIVE OUTCOME FROM OAK WILT

Working with County Extension Agents and County Horticulturists, plant pathologists have been able to get numerous articles in local and regional papers. Many of these papers have a circulation of several hundred thousand readers. Television and radio have also been used to create public awareness of the disease and what can be done to control its spread. This interest in a tree disease has significantly raised

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the public awareness for disease and pest problems and how they impact our environment.

As a result of the oak wilt management program, several businesses have been established to assist property owners in the control of this disease. These play an important role by making services available to individuals that otherwise would not be able to carry out a control program. As well, new economic opportunities are available for trained professionals.

Finally, as a result of the oak wilt management program, a group of individuals has been identified in Texas that is interested in trees and is willing to work to the betterment of all trees.

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Urban Oak Wilt Management in Austin, Texas

John Giedraitis
Elizabeth Drozda
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Oaks are the principal component of Austin's urban forest. The oak wilt epidemic (caused by *Ceratocystis fagacearum*) has killed more than 10,000 high-value oaks within Austin's city limits. Oak wilt has had a severe impact on residential property values, energy conservation, and other benefits associated with urban trees. A discussion of the value of trees in Austin, their importance to the environment and economy of the city, and their special place in the hearts of Texans is followed by an overview of oak wilt in Austin from 1934, when the disease was first noted, through 1985, when positive identification of the disease was made and efforts at management began. Austin's response from 1985 to 1988 is described, including how the community became educated about the epidemic, addressed public policy issues, and developed an oak wilt management plan. Neighborhood oak wilt suppression efforts since 1988 provide a model for partnerships between government and citizens to manage the disease. A brief summary of oak wilt program accomplishments is given.

AUSTIN: THE SETTING

Austin, the state capital of Texas, is the fifth largest metropolitan area in the state and is physiographically and culturally distinct from other Texas cities. About 750,000 people live in the Austin area, 466,000 of them within the city limits. During the most recent population "boom" from 1980 to 1986, Austin's population increased by one-third. Only about one-half the adult residents have lived in Austin more than 10 yr.

More than one-half of the adults in Austin are under age 35, making the city's population one of the youngest in the country. Austin's citizens are also among America's most educated; over 64% of adults have a college background, 33% having graduated with a degree and 31% having had at least one year of college (Garretson et al. 1988). Austin is home to the University of Texas, which has more than 50,000 students. The city also houses state government and numerous high-technology businesses.

THE VALUE OF TREES IN AUSTIN

When Austin was founded in 1839 as the capital of the Republic of Texas, it was chosen as much for its abundant resources of water, rock, and timber as for its great natural beauty (Horton 1839). Today, visitors approaching Austin by air are impressed by the nearly continuous canopy of green over the city. In fact, a 1991 University of Texas study found that more than 33% of Austin was under tree cover--a higher percentage than in any other city in which a similar study has been done (Crownover 1991).

Austin's location (30° north, 98° west) in central Texas marks the union of four different physiographic regions, including the Edwards Plateau (an area of rolling limestone hills), the Rolling Prairie, the Blackland Prairie, and the terraces and floodplains of the Colorado River. As a result, Austin enjoys an unusual diversity of terrain, soil, flora, fauna, and other natural resources. Oaks are the predominant tree species in this humid subtropical region. They survive the long, hot summers, which average 100 dys. each year over 90F°. Oaks also survive drops in

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temperature in the winter months (38° to 40F° average, with lows of 0F° on occasion) and an average rainfall of almost 32 in (City of Austin 1987).

Because oaks survive and even prosper in this sometimes difficult climate, and because they are long-lived and frequently associated with human events, Texans have developed a special fondness for them. For example, in Austin several live oaks (*Quercus virginiana* Mill. and *Quercus fusiformis* Small) have earned special recognition. The Auction Oaks still stand in downtown Republic Square Park, where in 1839 Judge Waller auctioned off the first city lots to raise money to build government offices in Austin. The Twin Oaks, two live oaks now located by the state capitol at the corner of Colorado and Fourteenth streets, are among the largest trees ever moved; it cost $45,000 to move them, not once, but twice in the past several years. Austin's own Treaty Oak, one of the most famous trees in the world, is the legendary site of the signing of a boundary treaty between Stephen F. Austin, the "Father of Texas," and the local Indians. Saved from developers in the 1930s, the Treaty Oak was poisoned in 1989, an event that attracted worldwide attention. Over $60,000 of the more than $100,000 spent to date to save it has come from H. Ross Perot, a wealthy Texas industrialist and onetime US presidential candidate.

Not only do oaks in Austin provide historical value and interest for tourists, but they also add value to that other abundant natural resource in Texas besides oil--real estate. In an effort to assess the financial impact of oak wilt (caused by *Ceratocystis fagacearum* [Bretz] Hunt) on property values in Austin, Texas A&M University (TAMU) evaluated the contribution made by trees to the sales price of homes. For 120 Austin home sites sampled, trees added between 13% and 19% of the sales value, with the mean lot value of trees set at $21,000. With live oaks at 28% and red oaks at 3% of the total trees sampled, the impact of loss from oak wilt ranged from 4% to 8% of the value of residential property (Martin et al. 1989). Given that residential property had an assessed value of $19 billion in 1992 (personal communication Debbie Strauss, Travis County Central Appraisal District), the loss of 4% to 8% in value could be between $800 million and $1.6 billion dollars (assuming all trees died). However, the impact could be even greater, because these trees are also some of the largest and most valuable trees on some residential sites.

The predominance of oaks, especially live oaks, from both favorable environmental conditions and selection by man, has set the stage for the oak wilt epidemic. Austin, with its abundance of oaks, is the city most heavily infected with oak wilt in the United States. The loss of more than 10,000 trees in the past 20 yr has had a tremendous impact not only economically but also aesthetically in a city where seven in 10 residents rank trees and landscape as very important or of the highest importance for the city's quality of life (Meyers 1985).

OAK WILT IN AUSTIN--AN OVERVIEW FROM 1934 TO 1985

In 1934, Taubenhaus first reported an apparently new disease affecting about 200 live oaks in Austin but did not learn its causes (Taubenhaus 1934). In 1965 Halliwell named the disease live oak decline (Halliwell 1965), and in 1970 Van Arsdel identified live oak decline as a vascular wilt that he believed was caused by *Cephalosporium diospyri* (Crand.) (Van Arsdel 1970). In 1972, the Austin newspaper reported that a rare disease was killing oaks on St. Edward's University campus in Austin, and the local cooperative extension agent correctly identified the problem as oak wilt caused by *C. fagacearum* (MacNabb 1972). By 1974, that proper identification had been recanted locally, so that live oak decline was considered the only official cause of oak mortality (Cox 1974). In 1977 the popular press was informing Austin citizens that researchers suspected that almost all live oaks in Texas, although they might not show symptoms of the disease yet, had already been infected with decline. In fact, the decline was believed to be a disease of Asiatic origin to which nearly every native tree was susceptible (Kilpatrick 1977). This misdiagnosis led to an emphasis on treatments such as experimental chemicals, pruning, and fertilizing, because prevention was not considered possible.

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In 1977, Dr. Robert Lewis correctly identified the cause of the epidemic in central Texas as oak wilt, and in 1979 he confirmed that oak wilt, not oak decline, was killing live oaks in Texas (Lewis 1977, Lewis and Oliveria 1979). Lewis disproved the misconception that the oak wilt fungus could not survive hot Texas summers, a belief that had hampered the proper identification. As Texas naturalist and writer John Graves noted, "In consequence of its habits, *C. fagacearum* was a pretty elusive felon, and to the layman it seems remarkable that anyone was able to track it down at all" (Graves 1984). Unfortunately, Lewis's findings were not immediately accepted by all officials.

Thus began the confusion that was played out in Austin for the next several years. From 1981 through 1985 the official diagnosis of oak decline caused Austin residents to invest tens of thousands of dollars on ineffectual fungicides and other unproductive treatments such as pruning (Associated Press 1981, Fisher 1985, Goodrich 1982, Szilagyi 1981).

AUSTIN'S RESPONSE TO OAK WILT 1985 TO 1988

In 1985, largely in response to the Austin City Council's concern over the many dead and dying trees on residential property and parkland, Austin hired its first city forester (the first author) within the Parks and Recreation Department (PARD) (City of Austin 1985). Although the scope of the position included public tree management, the top priority was to develop an effective oak wilt management plan. The forester's first objective was to determine if the problem was oak wilt, oak decline, or both. Through a series of interviews with experts around the state, a clearer picture began to emerge: Austin had a big problem.

At TAMU, a research team led by Dr. David Appel had been studying oak wilt and its management. In 1982, the Texas Forest Service initiated an oak wilt demonstration project with financial support from the USDA Forest Service. As part of that project, aerial photographic surveys established that oak wilt was the most serious disease of live oaks in central Texas and that there were dozens of oak wilt centers within the Austin city limits (Appel and Maggio 1984, Henigson 1984). By 1986, experts were in agreement that effective prevention and suppression treatments were now possible. The next step was to see if the suppression treatments recommended in Minnesota or Pennsylvania would work in Texas.

As information was collected on the nature of the epidemic and its spread, the City of Austin Oak Wilt Management Plan was initiated. The plan had five major elements:

1. Public education
2. Disease inspection and identification
3. Neighborhood surveys
4. Experimental chemical and mechanical controls
5. Formation of a city-wide task force

The first element, public education, began in February 1986 and to date has generated more than 100 news spots (e.g., Austin American-Statesman 1986a, 1987b, Nova 1986a, 1986b, Nova and Lewis 1986). In addition, talks were given to more than 30 neighborhood groups in 1986; these meetings frequently had more than 100 people in attendance. In 1987 a group of graduate students in the Radio, Television and Film Department at the University of Texas assisted in public education efforts by producing a 20-minute video entitled *Oak Wilt in Central Texas*. This video has played repeatedly on local cable access and municipal cable channels and reached a wide audience. Copies of the video also were made available at the forestry office for loan to individuals or groups.

This outreach led to a flood of calls for assistance from the community, particularly from affected neighborhoods and residents, and the second element of the management plan--disease inspection and identification--began. PARD received more than 300 inspection requests in 1986 alone. Based on inspections, a map of the city indicating all known or suspected oak wilt centers was created. Twenty-seven centers had been confirmed by 1987, 38 by 1988, 51 by 1990, and 72 by 1992 (Delgado 1987, Johnson 1988, Stanush 1990). Confirmation was either by visual symptoms--particularly veinal necrosis on live oaks--or by the preferred method of laboratory diagnosis.

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By July 1986, PARD had begun the third element of the management plan, neighborhood surveys. Several temporary forestry technicians were hired. They were outfitted with maps from the city Public Works Department and with microcomputers to input tree information. They were sent into the field to map every oak--whether healthy, sick, dead, or a stump--in the most visible oak wilt centers. By the end of the year, more than 20,000 trees had been mapped and recorded in 12 major oak wilt centers (Nova 1986b). Not only did these surveys form the basis for understanding the overall extent of oak wilt, but because every sick and dead tree or stump had been dated as to when the symptoms first occurred, the surveys also established an invaluable data base on rate and direction of disease spread. They indicated that the disease had been rapidly expanding both in numbers of new centers and along existing mortality fronts since 1981. Oak wilt centers in which more than 4,000 trees had died were found in individual neighborhoods (Delgado 1987, Smith-Rodgers 1986, Weingarten 1988b). These surveys would become the basis of neighborhood oak wilt management plans.

The fourth goal of the plan was to conduct research into more effective chemical and mechanical controls. To accomplish this goal, PARD entered into a cooperative agreement with TAMU, the Texas Forest Service, and the University of Texas at Austin. With TAMU, the City has cooperated on several projects. The first was on modeling disease centers to determine the rate and direction of disease spread (Nova 1986a). The neighborhood surveys done by PARD were used in this research, which determined the annual rate of tree-to-tree spread to be about 75 feet. Also in cooperation with TAMU, PARD has injected about a dozen trees with thiabendazole and more than 70 high-risk trees with propiconazole. Based on the promising results of the latter fungicide, its manufacturer, Ciba-Geigy Corporation, was granted registration to sell it in Texas to combat oak wilt. The product, called Alamo™, is now used as both a therapeutic and a preventative treatment (Johnson 1987, Slom 1987, Stanush 1990). Technical assistance from TAMU pathologists also was invaluable for the correct placement of trenches, a control method that was highly experimental in 1986 and 1987 (Austin American-Statesman 1986b).

The City also has conducted research with the Texas Forest Service, including oak replanting experiments and research on insect vectors of oak wilt. In 1986, the City and the Texas Forest Service jointly planted 42 trees within an oak wilt center in the Travis Heights neighborhood to test whether the fungus infects planted oaks (Nova and Lewis 1986). With the University of Texas, PARD has cooperated with Dr. Garry Cole to help fund and develop an oak wilt diagnostic test kit (see Silverman et al., these proceedings).

The last element of the City's management plan was to form the Oak Wilt Task Force. In November 1986, the task force members were appointed by the city manager. The members were citizens representing various groups, including neighborhood associations, the Austin Chamber of Commerce, the Central Texas Arborist Association, the Austin Board of Realtors, and the Austin Area Garden Council. Also included were a real estate law professor from the University of Texas, a former City Council member, and several at-large members. To help the task force, an advisory committee also was formed, with representatives from TAMU, the University of Texas, Texas Parks and Wildlife Department, the Texas Agricultural Extension Service, and PARD (Collier 1986).

The task force began meeting in December 1986 with a specific charge from the city manager: "to gather facts and opinions you require from experts, advisors, concerned members of your groups, and others concerned with oak wilt control and formulate a series of recommendations on disease control issues for the City Manager to present to the Mayor and City Council for their review and action." Key issues that the board was requested to review were property values, tree removals, red oak removal, trenching, education, research, and the role of the City in assisting neighborhoods (City of Austin 1986). Within one year, the task force had met, heard testimony from experts and citizens, and developed an eight-page recommendations report.

Two major accomplishments resulted from the task force's work. First, the recommendation that the task force should continue as a City Council-

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appointed board known as the Urban Forestry Board to investigate other tree-related issues was completed by ordinance in 1988 (City of Austin 1988b, McCann 1988a). Second, the City Council passed a diseased-tree ordinance requiring property owners to remove firewood or diseased trees officially declared to be a public nuisance (City of Austin 1988a, McCann 1988b). For its work, the task force received a statewide Urban Forestry Award from the Texas Urban Forestry Council (West Austin News 1987).

NEIGHBORHOOD OAK WILT SUPPRESSION EFFORTS IN AUSTIN 1988-1992

As the City's Oak Wilt Management Plan evolved, its focus shifted from inspections, education, and surveys to suppression of the disease spread in individual disease centers. PARD's first suppression plan was in the Travis Heights neighborhood. More than 704 oak trees, 200 of which had been infected within the last six years, were inventoried and mapped in the infection area. These trees and their symptoms were then recorded on a map that included recent utility line trenching. Once these data were analyzed, a series of recommendations was made and presented to the local neighborhood group (Giedraitis 1987). Much to the City's disappointment, the local neighborhood group would not, or could not, take action. With hindsight, the City recognized that it lacked experience in how to join residents together and help them to solve their own oak wilt problem.

In the summer of 1987, it became clear that the neighborhood group was not the vehicle for the implementation of the plan, so several activists within the neighborhood met to plan a strategy to raise enough money to fight oak wilt. Thus was born the Live Oak Festival. Led by two residents, Marc Dominus and Dale Stanka, the Travis Heights neighborhood set this goal: "To have a festival to get area residents and businesses together to learn more about the neighborhood's assets and problems, and to get acquainted and have a good time in the process. Our special emphasis will be on our beautiful oak trees. We hope to make participants more aware of the problem of oak wilt and establish a fund that will go towards research and helping people take care of their own oak wilt problems now by removing dead trees and trenching to stop the disease" (Tucci 1987). By October 1987, hundreds of residents had volunteered to produce a two-day festival. In the neighborhood park on Saturday, events and booths combined art, music, education, and the outdoors in support of the environment; on Sunday there was a tour of significant homes in the neighborhood. In all, the festival generated considerable enthusiasm, publicity, and over $5,500 (Breaux 1988). The Live Oak Festival was such a success that even though the Travis Heights treatment plan was implemented in February 1988, the festival has become an annual event (Taylor 1990, Weingarten 1988a).

By 1988, sufficient public support had been generated by neighborhood leaders, such as Barbara Cilley and others, that US congressman J. J. "Jake" Pickle took the area's concern over the epidemic to Washington, D.C. Through a series of communications with the chief of the USDA Forest Service and the testimony and support of Representative Pickle and others from the Texas delegation, Texas was awarded $170,000 from the USDA Forest Service for the Texas Cooperative Oak Wilt Suppression Project (Austin American-Statesman 1987a, Weingarten 1987). The federal-state cooperative project is designed to reduce the spread of oak wilt disease in selected counties of central Texas and within Austin and is administered by the Texas Forest Service (see Cameron and Billings, these proceedings).

Within the City of Austin, the Texas Cooperative Oak Wilt Suppression Project operates as a partnership consisting of the individual property owners who are affected by the disease, their neighborhood organization, PARD, the Texas Forest Service, and the USDA Forest Service. The project shares with landowners the costs of treating oak wilt centers to suppress the disease. The federal share provides up to 50% of the direct control costs, up to $5,000 per infection center or $5,000 per individual landowner. Priority is given to those landowners who are most interested in helping themselves and to well-defined centers that have rapid expansion and for which treatments are most likely to succeed (Texas Forest Service 1990).

When an affected landowner's property is within Austin's city limits, the landowner contacts the PARD

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oak wilt forester, who inspects the trees, confirms the presence of oak wilt, and prepares a written treatment plan. After the landowner, or in most cases the neighborhood group, approves and signs the treatment plan, the forester submits a request for cost-shares to the Texas Forest Service. Upon approval, the forester assists with getting the recommended work done. When receipts for specified treatments are submitted, the landowner is reimbursed for up to 50% of costs. Costs of particular treatment plans are based on the lowest bid from qualified contractors or on a flat rate established by the Texas Forest Service. Eligible treatments that can be cost-shared include the following:

- -- Trenching to sever common root systems around established oak wilt centers
- -- Removal and disposal of oak wilt-infected and selected adjacent oak trees
- -- Silvicide treatment or removal of individual red oaks having oak wilt symptoms
- -- Injection of healthy trees located inside the control trench with the fungicide Alamo®

With success of the efforts in Travis Heights, and shortly thereafter in the Stratford Drive, Edgemont Drive, and Northwest Hills areas, PARD developed a model for urban oak wilt suppression (Weingarten 1988c, 1989, Young 1988). The federal cost-share program assisted these efforts by providing financial incentives to help solve a common problem. Although it is certainly not easy to control oak wilt in a city, it is rewarding to see citizens join with their government to solve problems.

To date, PARD has assisted in 22 oak wilt suppression plans, and with each one, implementation methods continue to evolve. Currently, an oak wilt suppression plan involves about 30 steps from the initial request for assistance from a resident or neighborhood group through conducting annual follow-up evaluations and treatments. These steps are outlined in Table 1.

The process is a complicated series of political, social, biological, bureaucratic, emotional, and economic steps and is usually too much for a single homeowner to accomplish. In every area where people have joined together and done something about oak wilt, it has been because one or a few leaders have managed to get things moving among the many participants in their neighborhood. The City helps all it can, but citizens have to be willing to make the commitment to be a full partner (Perry 1988). These people serve as the liaisons so that instead of guiding numerous individual homeowners through each step, PARD deals with one or a few leaders. This perspective is useful because a neighborhood plan is a unified procedure in which homeowners must join together and agree to a common action to suppress the disease and qualify for cost-share rebates.

PROGRAM ACCOMPLISHMENTS

Currently there are 72 confirmed oak wilt centers in Austin. Treatments, including trenching, tree deadening and removal, and/or fungicide injections have been completed in 22 centers. Over $137,000 has been spent by Austinites on residential property to fight oak wilt under the Texas Oak Wilt Suppression Project. Some 14,057 ft of containment trenches have been installed, 500 trees have been removed, and 62 trees have been injected with fungicide. Trenching costs have ranged between $1.50 to $11.00 per linear ft, depending on soil conditions, man-made structures, utilities, permits, and so forth. The average cost to remove an infected tree is about $109. The City's commitment to funding has remained fairly steady since 1989 at about $60,000 for one full-time and one temporary position, $25,000 of which is refunded from the Texas Oak Wilt Suppression Project.

Although there have been a number of remarkable accomplishments in neighborhood suppression projects, there also have been a few notable frustrations. But as is repeatedly pointed out to skeptics of urban tree disease management, to take no action is to suffer accelerating losses of the main feature of Austin's urban forest. Such losses are not acceptable in Austin. Oak wilt management is an evolving art and science. Austin, with its magnificent oak forest, is committed to remaining at the forefront of efforts to prevent and suppress oak wilt.

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Table 1. Steps taken, after oak wilt has been detected, by the PARD oak wilt specialist in a neighborhood oak wilt suppression project in Austin, TX (1992).

1. Request for assistance from resident and/or neighborhood group.[1,2]
2. Assign the work area a priority.[1]
3. Obtain base maps.[1]
4. Make work copies of maps.[1]
5. Contact neighborhood leaders and explain that the survey is beginning and they need to start their fund raising and homeowner education efforts.[1]
6. Inform homeowners of oak wilt in the area - usually through the local paper.[1]
7. Neighborhood organizers send a letter to the mayor expressing interest in participating in a cooperative project with the city.[2]
8. Prepare a response for the mayor.[2]
9. Survey the area by mapping trees and recording data and observations.[1]
10. Monitor field work.[1]
11. Color-code oak wilt infected and fungicide injected trees on the map.[1]
12. Conduct final field evaluation.[1]
13. Mark 100 foot barrier on the map.[1]
14. Draft copy of the Suppression Plan to the neighborhood leaders.[1]
15. Have neighborhood leader(s) design the Texas Forest Service cost share forms.[2]
16. Record total diameters of trees within the barrier for Alamo™ fungicide injections.[1]
17. Revise plan /develop final plan.[1]
18. Prepare cost share applications.[1]
19. Raise funds to pay for treatments.[2]
20. Send cost share applications to the Texas Forest Service.[1]
21. Develop bid specifications for treatments for the neighborhood group.[1]
22. Help neighborhood collect bids.[1]
23. Bid, hire and supervise a contractor.[2]
24. Monitor contractor.[1]
25. Conduct final inspection.[1]
26. Pay contractor.[2]
27. Provide receipts for reimbursement(s).[2]
28. Process paperwork for reimbursement of the cooperator.[1]
29. Receive cost share funds from the Texas Forest Service.[2]
30. Enter data into computer base.[1]
31. Conduct post-suppression evaluations bi-annually to determine effectiveness of treatments.[1]
32. Re-treat as necessary to control breakouts (start back at number one).[1,2]

[1]Action step for the Parks and Recreation Department, Urban Forestry Program, Oak Wilt Suppression Unit.
[2]Action step for the neighborhood group.

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Section V
Future Direction of Oak Wilt Programs in Texas

Future Direction of Oak Wilt Research at Texas A&M University

David N. Appel

Oak wilt in live oaks may successfully be controlled in Texas by the implementation of currently recommended management techniques. However, these recommendations sometimes fail to save valuable trees, even where a program is apparently implemented properly. Failure to control oak wilt is attributed to a limited understanding of pathogen spread and response of the host to infection. Four experimental approaches are being taken in the Forest Pathology Laboratory at Texas A&M University to address gaps in our knowledge of the epidemiology and control of oak wilt. The specific approaches include studying; 1) the responses of new hosts to infection by the oak wilt fungus, 2) the distribution and persistence of the fungicide propiconazole in infected trees, 3) the population biology and spread of the pathogen, and 4) the survival of the pathogen in roots of trees. The rationale for each of these studies is discussed, together with a brief description of specific objectives and methods.

INTRODUCTION

Since 1981, research in the Forest Pathology Laboratory at Texas A&M University (TAMU) has focused on the cause of widespread live oak mortality in central Texas and how losses of valuable trees can be reduced. Initially, aerial surveys with color infrared photography was used in portions of central Texas to locate diseased trees for ground inspection and diagnosis (Appel 1986, Appel and Maggio 1984). Oak wilt was determined to be the major cause of mortality in the live oak population, particularly in the Kerr-Bandera, Lampasas-Burnet, and Travis county areas.

In addition to these extensive survey efforts, components of the oak wilt epidemic and characteristics of the pathogen, *Ceratocystis fagacearum* (Bretz) Hunt, were subsequently studied in Texas and compared to pathogen behavior outside of the state. Specific components included potential insect vectors (Appel et al. 1986, Appel et al. 1990), rates of foci expansion (Appel et al. 1989), inoculum formation, mating type distribution (Appel et al. 1985), fungal pathogenicity, and host susceptibility (Appel 1991, Appel et al. 1987, Peters 1985). This comparison between oak wilt in Texas and the disease elsewhere also included control methods developed for deciduous oaks in other states and their application to management of oak wilt in live oak (Appel and Lewis 1985). The emphasis in testing control methods was on disruption of root connections, roguing, and intravascular injection with fungicides (Appel and Kurdyla 1992).

Oak wilt losses in Texas can now be prevented under most conditions where the disease occurs (Appel 1991, Appel et al. 1990, Johnson and Appel 1984). Various combinations of trenching, tree removal, and injection with propiconazole are sufficiently successful to warrant their recommendation to successfully reduce losses from oak wilt in live oak (Johnson and Appel 1989). However, there are still gaps in our knowledge of the

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disease resulting in occasional failures to manage losses at acceptable levels. These failures are most notable when the fungus grows across a trench, or when a healthy tree at high risk of infection is injected preventively but eventually succumbs to the disease.

The lack of disease control does not always reflect a limitation in the technology being recommended. The successful application of many control techniques often requires training, judgement, and far more experience than is normally available to the non-professional. When expectations for control are not met, there is a loss in confidence that requires clear explanations of plausible reasons for failure; in many cases, the cause of failure is unknown. Also, changing regulatory policy and public attitudes toward environmental issues necessitate further research to maintain a flexible program to control the disease. For these reasons, additional research is required to further clarify our understanding of the disease so that we may progressively improve our management approach.

Research on oak wilt at TAMU will be focused on four general areas in the immediate future. These include; 1) variability in host response to infection by *C. fagacearum*, 2) the uptake, distribution, and persistence of propiconazole in injected trees, 3) the utilization of genetic markers to better understand long distance and "local" transmission of the pathogen, and 4) the survival of the pathogen in the root systems of symptomatic and asymptomatic trees located throughout active oak wilt centers. The rationale and experimental approach for each of these topics is discussed briefly below.

VARIABILITY IN HOST RESPONSE TO INFECTION

Rationale

There are between 200 and 300 species of *Quercus* growing in temperate regions of the northern hemisphere, at high altitudes in the tropics south to Columbia in America, and through the Malayan Archipelago in Asia (Irgens-Moller 1955). As far as we know, the oak wilt range extends through only a small portion of the potential host range and has challenged only a minority of known *Quercus* species. This is particularly true of the live oaks which have their principal areas of concentration in the southwestern U.S., Mexico, and Central America. The rich species diversity within *Quercus*, virulence of *C. fagacearum*, and apparent limited distribution of the pathogen provides the opportunity to derive a great deal of information from the screening of "exotic" oaks for disease susceptibility.

Another potential source of host resistance may be found in existing oak wilt centers in central Texas. Growing within most actively expanding disease centers are varying numbers of debilitated, surviving live oaks (Appel et al. 1989). Acorns are being collected from these types of trees to test their progeny for response to infection and colonization by the pathogen.

In a different approach, the basis for survival has been studied using electrophoresis of isozymes to examine genetic variation in the pre-epidemic and post-epidemic live oak populations. In these studies there was presumed to be a correlation between isozyme phenotype and tree survival (Bellamy et al. 1992). Although no definitive correlation was revealed, there were population differences in allelle frequencies and heterozygosity between survivors and unchallenged trees. These differences may be the result of natural selection operating during the passing epidemic and warrant further investigation.

Results of these studies could identify sources of resistance to *C. fagacearum* so that efforts could be undertaken to produce improved oak selections. The potential for oak wilt epidemics in regions as yet unchallenged by the pathogen could be more accurately assessed. Finally, a better understanding of pathogenicity and virulence in the *C. fagacaerum* population would be achieved.

Methods

The techniques used to screen *Quercus* species for resistance and susceptibility to the oak wilt pathogen are based on previous inoculation studies by Fenn et al. (1975) and Haynes (1976). They have already been used to study responses in live oak to artificial inoculation with diverse *C. fagacearum* isolates (Peters 1985). Consistent, reliable disease response

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can be obtained by introducing small quantities (10 - 20 µl) of a spore suspension (1 x 10^6 spores/ml) into wounds made on the main stems of young (6 mos. - 5 yrs.), containerized trees. Trees may be held in growth chambers or greenhouses following inoculation; disease response usually occurs within a month following inoculation, and may be followed for up to a year depending on the size of the tree and objectives of the study. Various parameters, including latent period, proportion of crown loss, and resprouting at the base of the inoculated tree have been found to be useful for measuring host response.

Various live oak species from diverse geographical regions will be obtained and inoculated using the standard methods described. One source of potentially important resistance is surviving live oaks located on the interiors of active oak wilt centers in central Texas. Acorns from those trees have been collected, germinated, and grown to the seedling stage for inoculation with *C. fagacearum*. These studies will be repeated and expanded to include other selections of live oak with desirable characteristics.

In addition to those live oaks within the current range of oak wilt in Texas, there are numerous species of live oak in West Texas that are as yet unchallenged by the fungus. Examples include scrub live oak (*Q. turbinella* Greene), Hinckley oak (*Q. hinckleyi* C. H. Mull.), and lateleaf oak (*Q. tardifolia* C. H. Mull.). These and other "exotic" *Quercus* spp. from outside the existing range of the disease will be collected as acorns, or as containerized nursery stock and inoculated for disease response.

Inoculations will be made with isolates of *C. fagacearum* having a known capacity for causing disease. These studies will benefit by adding different pathogen isolates so the potential pathogenicity in the *C. fagacearum* population can be determined. Inoculum types, concentrations, and growing conditions will all be standardized for comparative purposes and to ensure that a reliable response is obtained.

INTRAVASCULAR INJECTION WITH PROPICONAZOLE

Rationale

As a result of research in the TAMU Forest Pathology Lab, tens of thousands of trees are being injected annually in Texas with propiconazole (Alamo®, CIBA Corp., Charlotte, NC) for oak wilt prevention and therapy (Dr. Jerral Johnson, personal communication). Homeowners, commercial arborists, and state agency personnel are utilizing injection to further their control efforts. Propiconazole is now being used nationally for treatment of oak wilt as well as for Dutch elm disease, caused by *Ophiostoma ulmi* (Buisman) Nannf. In spite of the popularity of the injection technique, there are many facets of the process that are poorly understood.

Tree injection is a complex process involving numerous anatomical, biochemical, and physiological interactions in the tree. Few of these processes have been studied, but it is known that there are natural barriers to pressure injection of liquids into the vascular system of a tree. Therefore, there is still an unacceptably high, unaccountable failure rate in trees that are seemingly good candidates for successful treatment.

Another source of uncertainty in the injection process is the necessity for re-treatment. Trees injected on a preventative basis may be challenged for several years by the pathogen spreading through their root systems. The success of treatment may depend upon substantial distribution of the fungicide in a large proportion of the xylem vessels. Much of this uncertainty could be alleviated by developing a reliable, simple experimental technique to assay for propiconazole and study the compound in the vascular systems of treated trees.

There are several variables that could easily be manipulated and have the potential to improve the chances of a successful treatment. These include time of year, concentration, and/or volumes of solution, locations of injection ports on the tree, pressure used to apply the solution, and formulation of the fungicide. At present, it is difficult to assess how these and other factors might influence the

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outcome of an injection, other than observing tree survival over a number of years.

An alternative method is to measure the uptake, distribution, and persistence of fungicide in injected tissues. Once these measurements are taken, those variables mentioned above can be manipulated and the outcome assessed. Presumably, presence of the fungicide in as great a proportion of host tissues for as long as possible will achieve the best chances for tree survival.

Methods

Two approaches may be used to assay tree tissues for the presence of propiconazole. The simplest is a bioassay. A crude extract of wood from treated trees is prepared, and fungicidal activity detected using thin layer chromatography. The extracts are developed on a silica gel chromatography plate that is sprayed with a nutrient suspension of conidia from the indicator fungus, usually a *Cladosporium* spp. Following incubation of the chromatography plate, blank spots appear where the fungicide has inhibited growth of the fungus. The size of the inhibition zone will reflect the amount of fungicide in the tissues with an acceptable level of precision. However, a more precise and sensitive measure of fungicide may be obtained through the use of high pressure liquid chromatography. Extraction conditions are more rigorous and the separation and detection methods far more precise than the bioassay. Using these two techniques, a better understanding of the uptake, distribution, and persistence of propiconazole in treated trees will be gained.

Injections will be conducted in the manner described in a previous report (Appel and Kurdyla 1992). The system will first be tested on small (5 - 10 mm dbh, 8 - 12 m tall) live oaks growing in a plantation established in 1983 on a TAMU farm in Brazos Co. Following injection, these trees will be uprooted and the exposed roots sampled to test for downward movement of the fungicide into root systems. To measure uptake and distribution of fungicides in crowns under realistic conditions, naturally occurring native trees will be injected seasonally. To measure persistence, trees treated experimentally in previously conducted efficacy trials will be dissected and assayed. Trees treated since 1987 are available for testing. Expanded testing will compare the high volume root flare injections with low volume microinjections for distribution in trees.

PATHOGEN VARIABILITY AND POPULATION BIOLOGY

Rationale

The spread of *C. fagacearum* has been studied at least as much as any other aspect of the pathogen's life cycle (Gibbs and French 1981). In spite of these efforts, instances still occur when current explanations for pathogen transmission are insufficient, especially when control measures fail. Outbreaks of oak wilt beyond a properly placed trench or in areas where no known inoculum sources exist are sometimes difficult to explain with our current understanding of the disease. Our knowledge of the origin of *C. fagacearum* in the U.S. also is limited, thus hampering our ability to predict where and how the pathogen will affect other *Quercus* spp. in areas outside the current range of the disease.

One experimental approach to these problems is to study the population biology of the fungus. This is one area of oak wilt research that has received little attention. With the exception of an albino mutant (Barnett et al. 1954), some minor morphological variation in culture (Barnett 1956), and the identification of mating types, *C. fagacearum* populations have not been characterized in detail and related to specific episodes of disease initiation, either under natural conditions or artificial inoculations. The relative roles of the sexual and asexual stages of the pathogen in the development of an epidemic are not known. This is particularly important in Texas where our knowledge of potential vectors is limited and host type has such an extremely important influence on reproductive stages.

Methods

Culture collections will be made from numerous populations of *C. fagacearum* at the local, regional, and national scales. On a local scale, collections will be made to investigate the pathogen population

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existing within individual trees as well as within individual disease centers. Isolates will be collected from asexually expanding populations in live oak presumably transmitted exclusively through connected root systems. These clonal populations will be contrasted with isolates taken from disease centers with live oaks where fungal mats are forming on red oaks and sexual reproduction is observed. On larger scales, isolates collected from throughout Texas will be compared with collections from Minnesota, West Virginia, and other states where culture collections have been made and maintained.

A recently developed molecular "marker" will be used to detect variation in DNA sequences on similar regions of chromosomes from each isolate in a collection (McDonald and McDermott 1993). This technique depends on the isolation and purification of DNA from each isolate; the DNA is then digested into fragments with "restriction enzymes" and size fractionated using agarose gel electrophoresis (Southern 1975). Probes, consisting of DNA sequences, are then hybridized to the fractionated fragments on which there is a sequence with homology to the probe. Variation in the DNA of pathogen isolates from presumably different populations then will be reflected in different hybridization patterns.

SURVIVAL OF *C. FAGACEARUM* IN ROOT SYSTEMS

Rationale

A majority of live oak mortality in Texas, as in other states, results from the transmission of *C. fagacearum* through functional root connections between diseased and healthy trees. For this reason, trenching to break those connections and thus prevent root transmission is one practical way to control the fungus. Root transmission also is an important factor in the success or failure of intravascular injection with fungicides.

Although the mechanism for successful control of the disease with intravascular injection is unknown, the survival of the pathogen in the root system of a challenged, treated tree is a critical factor in the long term efficacy of the treatment. Little is known of the response of roots on a tree being challenged by the oak wilt pathogen, but it would appear that colonized roots remain viable and harbor the fungus for years following the death of the tree crown and trunk.

Methods

These experiments will be located in areas planned for root trenching and tree removal under the supervision of the Texas Forest Service and the guidelines of the Texas Oak Wilt Suppression Project. Roots will be removed from trenches dug to control the disease and from trees uprooted for removal and destruction. The sites will be mapped prior to treatment and all samples recorded as to position in the field. In addition, trees will be rated for symptoms and distances between diseased and healthy trees will be recorded. Where possible, secondary trenches will be dug to gain access to roots growing in gaps between trees where there is interest in the potential for root transmission of the pathogen.

All root samples will be stored on ice in the field and returned to the laboratory for attempted isolation of *C. fagacearum*. Soil also will be collected and processed to test for survival of the pathogen as a free-living saprophyte. Root isolations will be attempted on acidified potato dextrose agar (APDA), while soil dilution plates will be made with antibiotic amended APDA.

CONCLUSIONS

Each of the experiments described above is designed to study a specific question concerning how the oak wilt fungus survives and spreads. Hopefully, findings from this research will provide new mechanisms for effective control. There are, however, some themes common to all of the experiments. Pathogen survival in root systems for example, a subject of the last objective, is relevant to every control technique currently recommended, with particular importance to trenching and injection. The long term success of each of these controls will depend on how long they persist under constant challenge by the pathogen as it grows through a root system. At present, we have no evidence to predict how long it might take for the fungus to die and render the tree permanently protected.

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Oak wilt has had a significant impact on the live oak savannahs and urban forests of central Texas and can be expected to be a permanent feature in the landscape. In addition, the fungus can be expected to spread to new areas within the state and will probably expand to new states over time. How long it may take for this expansion to occur cannot be reliably predicted, but we do know that as the fungus encroaches into new forest ecotypes, unforeseeable problems will arise when attempting to control the disease. At present, management options are limited, but effective, when properly implemented. The need for further research is not only an important priority for alleviating the destruction currently being sustained in Texas, but for reducing the rates of future epidemics that are certain to occur.

The response of the agricultural and natural resource management community in Texas to the oak wilt epidemic in Texas has gone through several stages. Initially, there was confusion concerning the cause of widespread oak mortality. Progress from that stage to the current application of widespread control efforts has depended on the results of reliable research. Continued research is needed if efficacy of our preventative and control efforts are to improve. The progress in controlling oak wilt in Texas also has depended on close cooperation among forest managers, extension personnel, and all of those responsible for the transfer of information and application of available technology to manage the disease. It is the intent of the Forest Pathology Laboratory at Texas A&M to continue to devise new studies to help in the efforts to reduce losses and preserve the integrity of Texas oak resources.

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Future Direction of USDA Forest Service Research

A. D. Wilson

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

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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

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live oak wood could be a potential source of inoculum. The role of red oaks deserves particular attention because these trees, although generally more 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 among 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

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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

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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.

ACKNOWLEDGMENTS

The author thanks Jim Solomon, Ted Leininger, and Harvey Kennedy for reviewing the manuscript.

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Future Direction of the Texas Forest Service Oak Wilt Suppression Project

E. H. Barron

The Texas Forest Service involvement in oak wilt began as a cooperative Demonstration Project with the USDA Forest Service in 1982. It was followed in 1988 by the current Suppression Project. Based on the continued strong interest by property owners, policy makers at the federal and state levels, and the favorable results obtained over the previous four years, the Oak Wilt Suppression Project has a definite future. State forestry programs in the near future will likely face some retrenchment as a result of budget difficulties at both the federal and state levels. Increased state or federal funding for oak wilt does not appear likely in the near future. However, new cooperative forestry programs initiated as part of the 1990 Farm Bill will enable the Texas Forest Service to leverage existing suppression funds to further expand the Texas Oak Wilt Suppression Project.

INTRODUCTION

The Texas Forest Service (TFS) began its pest control program in the early-nineteen sixties with a program to assist timber growers of East Texas in addressing the southern pine beetle problems that caused, and continues to cause, serious economic losses. This agency, for most of its seventy-six year history, focused on protection and development of the forest resources of East Texas. In the mid-seventies, TFS began to expand into other regions of the State with a windbreak program for the High Plains, a small urban forestry program, and a program to assist volunteer fire departments. Therefore, when the TFS became involved with assisting landowners in central Texas with oak wilt suppression, the agency did not have the resources in place in this region of the state to adequately deliver the Project.

In 1981, a small group of landowners in Bandera County, led by Mr. "Speedy" Hicks and then Congressman Tom Loeffler, invited the officials of the Texas Forest Service and the USDA Forest Service to their area to review the oak wilt problem and to discuss possibilities for assisting landowners with combating the loss of the oak forests of central Texas. Dr. David Appel, of the Texas A&M Agricultural Experiment Station, was a participant in the early formation of this cooperative project. This was the beginning of the Oak Wilt Demonstration Project, which Dr. Scott Cameron discussed in his presentation which provided the ground work for the Suppression Project that followed.

Dr. Robert Lewis, with the USDA Forest Service research group in Stoneville, Mississippi, while working at the L.B.J. Ranch in Texas, had previously confirmed that the causal organism was *Ceratocystis fagacearum* (Bretz) Hunt. Dr. Appel had demonstrated some success with experimental control treatments. The Demonstration Project helped TFS determine where to focus assistance and how to

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design an effective control program. Initially, it was quite evident that in some areas the disease had become so extensive that reforestation was needed more than disease control. Therefore, one objective of the Demonstration Project was to explore reforestation opportunities with tree species that were native and either resistant or tolerant to the disease. Dr. Brad Barber, of the Texas Forest Service, coordinates this research.

The federal government made available approximately $25,000 annually for the Demonstration Project. Some of the TFS staff from the Pest Control Section and our Department, along with Dr. Appel and Dr. Robert Maggio from the Texas A&M Agricultural Experiment Station, contributed time to the project.

In 1988, the USDA Forest Service provided approximately $168,000 in federal funds to initiate a cooperative Oak Wilt Suppression Project. Matching funds were required from the state and its cooperators. This presented a challenge to the agency since most of our employees were needed in East Texas and could not be spared to move to central Texas. Although the federal government provided money, the state did not increase funding for this new project. The TFS decided the project had merit and should be implemented as best as possible within existing resource constraints.

The TFS reassigned two urban foresters to work on the project part-time; one based in Fort Worth and one in San Antonio. We moved a full-time professional position from our Pest Control Section in East Texas to Travis County. We hired seasonal technicians to support these positions. The City of Austin had become very concerned about the loss of trees within the city and agreed to share the cost of a forester and technician for the project. Technical supervision was coordinated by our staff in Lufkin and the project was administered by our College Station staff.

Later, a program specialist and two additional foresters were hired and stationed in Austin, Meridian, and Kerrville, respectively, to better serve the central Texas region. Office space was provided by the cities of Fort Worth and San Antonio, the USDA Agricultural Research Service, and Travis County. County staffs of the Soil Conservation Service and the Texas Agricultural Extension Service have been very instrumental in helping oak wilt foresters connect with local landowners. Without their familiarity with local situations, we could not have experienced the early success we had in reaching strong cooperators.

FIRST FIVE YEARS OF OAK WILT SUPPRESSION

Clearly, the first five yr of the suppression project have been a learning experience. The project has provided a valuable foundation for evaluating the impact of the disease, public interest and support for control, and, perhaps most importantly, information on the effectiveness and benefit: cost ratio of direct suppression methods under Texas' conditions.

As you have heard from previous speakers, despite a small work force, project accomplishments have been considerable:

- more than 300 disease centers controlled
- more than 100 miles of trenches installed
- more than 1,700 trees injected with fungicide
- more than 1,000 infected red oaks removed

As McKinney and Billings reported (these proceedings), the project is supporting an economic analysis that reveals a very positive benefit:cost ratio for cost-shared suppression treatments. This is particularly true in rural areas where trenching, which is a large part of the cost, is an effective treatment in halting the spread of infection centers. Even in highly populated urban centers where control costs are much higher, the benefits still exceed the costs due to the tremendous value of live oaks to urban landscapes in central Texas.

One of the most important measures of success of the project is that 80% of the treatments installed to date have been successful in stopping the spread of the disease. This is especially important in light of the $900,000 being spent annually. Of this total, property owners are spending at least $220,000, the State $200,000, and the federal government $450,000. In addition to this is the money spent by the City of Austin and the many cases where controls are implemented entirely at property owners' expense.

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LEVERAGING FROM NEW PROGRAMS

The 1990 Farm Bill has significantly improved the ability of the TFS to meet the needs of landowners in all parts of Texas. Of most importance to this program, the Act authorized and funded the Forest Stewardship and the Urban Forestry Programs. These two programs compose President Bush's America the Beautiful Program which has a goal of planting a billion trees annually.

The Forest Stewardship Program is the rural component that provides landowners with technical assistance and cost shares to increase forest cover and improve existing forests to enhance environmental values as well as timber production. To receive financial assistance, a landowner must demonstrate good stewardship management. The program is applicable to all parts of the state having tree cover. It includes preparation of stewardship plans and practices that address reforestation, water quality, wildlife habitat, wetlands, endangered species, windbreaks, and improving existing stands of trees. From the standpoint of oak wilt, for the first time we will be able to technically and financially assist with reforestation of areas impacted by the disease. The Suppression Project could only assist with control of the disease.

The America the Beautiful Program provides the State Forester with added resources from which to service the millions of acres of additional forest land being impacted by oak wilt. It is very difficult for one program to bear all the costs of a cooperative forestry program over an area as large as central Texas. In Texas for fiscal year 1992, we received $415,000 to provide technical assistance under the Forest Stewardship Program, and $530,000 to share up to 50 percent of the cost of installation of rural forestry practices with landowners. Keep in mind that this program covers the entire state; however, in central Texas the program is focused on oak wilt sites.

The Urban Forestry Program also received a major boost in the 1990 Farm Bill. In Texas our federal funding for this program went from $30,000 to $442,000 per year. This enabled the agency to expand full-time technical forestry assistance to four regions of the State that were not previously serviced. Almost half of these funds are used to strengthen the urban forestry programs of our local cooperators. Leveraging the limited public dollars is extremely important in effectively addressing the huge cost involved in planting and caring for trees in the urban environment. For the past two years, we also have received almost one million dollars per year from the Small Business Administration to use for matching grants to help local cooperators plant trees on lands owned by local governments.

We are very excited about the added capabilities these new programs have provided. They allow us to address reforestation needs in central Texas in a meaningful way and the opportunity to provide professional staffing with multi-program responsibilities on more of a state-wide basis.

FUTURE DIRECTION

Another new program that will likely be in the Texas Forest Service future is the National Forest Health Monitoring Program. This is a cooperative program involving State Foresters, the USDA Forest Service, and the Environmental Protection Agency, and is part of EPA's Environmental Monitoring and Assessment Program (EMAP). This is an interagency program to monitor the condition of the nation's ecological resources. The public is increasingly concerned that the resources they rely upon for recreation, quality of life, and economic livelihood remain sustainable. The National Forest Health Monitoring Program will attempt to assess where and at what rate these resources may be degrading. Forests are one of the seven resource groups to be monitored.

This program will help raise the awareness level of the full impact that oak wilt has on the forest resources at both state and national levels, and hopefully, will bring additional financial resources for comprehensively evaluating losses to the disease.

Federal funding for fiscal year 1993 is still under debate in Congress. According to Congressman Jake Pickle's staff, the House Interior Appropriation Subcommittee has recommended full funding for the Oak Wilt Suppression Project. However, the Administration had not budgeted for it. On the positive side, the Administration did provide for substantial increases in the Forest Stewardship

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Program, the Stewardship Incentive Program, and the Urban Forestry Program. If these increases survive the budget process, it will significantly boost the reforestation efforts in central Texas and enable us to further strengthen the urban effort, all of which complement the Oak Wilt Suppression Project.

Although this is an election year, Congress appears to be responding to the public's growing concern over the budget deficit. An indicator that they may be willing to make tough budget decisions was the defeat of funding for the $8.25 billion Superconducting Collider. Therefore, we are cautiously optimistic about our federal funding.

State funding for the next biennium is also very uncertain. This past week, as an indicator of the mood of legislators, top Texas policy makers stated that no new state money will be budgeted for public education in the next legislative session. Indeed, budget cuts are very possible.

In the near future, we hope to establish two new positions; one at La Grange to extend the Oak Wilt Program to Fayette and Lavaca Counties, and one to better serve the Llano, Burnett, and Lampasas area.

We have just initiated a project to evaluate the utility of aerial videography for surveying oak wilt damage. Once procedures have been developed and refined, a survey will delineate the severity of oak wilt throughout central Texas. This, combined with the final results of the economic analysis of the project, will allow us to more effectively target our efforts in the project.

In the long run, we hope to establish a central Texas field station, similar to our Pest Control Laboratory in Lufkin. This field station will include staffing to administer the Oak Wilt Project, conduct the Forest Health Monitoring activities for the region, leverage the on-going research activities, provide diagnostic capabilities for oak wilt and other tree-related pest problems, and provide additional support to the Urban Forestry Program.

Emphasis will be placed on developing an effective delivery system for the reforestation program in central Texas. This includes developing a seedling supply of the appropriate species, refining technical guidelines, and establishing a private vendor system to conduct site preparation and tree planting for landowners.

For landowners to be eligible for cost share assistance for tree planting, they must have a Forest Stewardship Plan for their property. Our foresters will give considerable time to this phase of the Project in the near future.

As part of the recent United Nations "Earth Summit", the leaders developed a set of Forest Principles that will give added support to our forestry programs in this country. The objective of the Principles is to achieve the management, conservation, and sustainable development of forests, and to provide for their multiple and complementary uses. One of the principles calls for national policies and strategies that provide a framework for increased efforts, including developing and strengthening institutions and programs that accomplish these objectives. With this kind of worldwide attention, I believe we can all look forward to more support for our forestry programs, at both the federal and state levels, in the years ahead.

CONCLUSION

Private property owners have been very supportive of the Cooperative Suppression Project as evidenced by their willingness to invest more than $200,000 annually of their own money in control treatments and by their volunteering of time to organize neighborhoods to fund and install control treatments. The high rate of success of the treatments and the high benefit:cost ratios for the program support a definite future for the project.

The project of the future will be a more integrated effort taking advantage of resources coming from new forestry initiatives. This will enable the project to offer a more complete package to address the needs of property owners impacted by the disease.

The project will benefit by the growing support of organized forestry groups like the National Association of State Foresters, the National Urban Forestry Council, the Texas Urban Forestry Council, the regional Urban Forestry Councils, and the State Forest Stewardship Council.

Continued success of the program is very dependent on continued support from our major partners including the USDA Forest Service, the

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Texas A&M Agriculture Experiment Station, the City of Austin, local volunteer groups, the Texas A&M Agriculture Extension Service, the Soil Conservation Service, and others.

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