1-1-1960

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BULLETIN 448T • DECEMBER 1960

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WEST VIRGINIA UNIVERSITY AGRICULTURAL EXPERIMENT STATION

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Introduction

CITIZENS of West Virginia are interested in the health of their forests since these cover nearly 70 per cent of this State's rugged terrain. We are all concerned when these forests are threatened by destructive insects and diseases. The loss of the American chestnut has made us aware of the danger.

It is natural for West Virginians to want to know about oak wilt, since oaks threatened by this disease now make up more than half of our standing timber. This bulletin is designed to present, in one place, information concerning the history of this disease, its nature, cause, present importance, and the prospects for its control.

Research scientists at the West Virginia University Agricultural Experiment Station have been investigating oak wilt since it was first found in West Virginia in 1951. This bulletin deals in part with our own previously unpublished information and in part with data presented by us and by others in more than two hundred published articles concerning oak wilt and related subjects. It also contributes to the evaluation of these data as parts of the unfolding story of oak wilt in the United States.[2]

Oak wilt is known to have caused serious losses among oaks of the red-oak group[3] (Hepting, 1955) (Figure 1), and today it threatens all oak species growing in the 18 states where its presence is recognized. (Figure 2, see map of U.S. attached inside back cover.) The erratic

[1]The authors are respectively, Plant Pathologist, Mycologist, Entomologist, and Plant Pathologist and Head, Department of Plant Pathology, Bacteriology and Entomology. They wish to acknowledge the helpful cooperation received from the West Virginia Department of Agriculture, the West Virginia Conservation Commission, the United States Forest Service and Soil Conservation Service and the Division of Forestry of West Virginia University. (Approved for publication January, 1960.)

[2]The current oak wilt situation in West Virginia is dealt with briefly and specifically in West Virginia University Agricultural Experiment Station Circular 112, *Oak Wilt and Its Control in West Virginia,* by R. P. True and W. H. Gillespie.

[3]Oaks closely related to red oak (*Quercus borealis* Michx.f.), including black oak (*Quercus velutina* Lam.) and many other species whose leaf lobes or leaf tips are pointed and terminate in a spine, make up the red-oak (Enythrobalanus) group. Members of the white-oak (Leucobalanus) group have the tips or lobes of their leaves rounded and spineless.

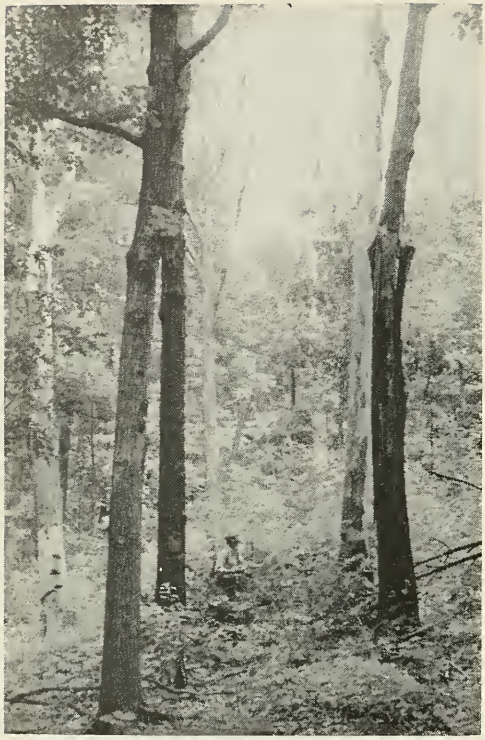


FIGURE 1. Views in a West Virginia forest affected by oak wilt. Left. The disease area. Right. Adjacent forest not yet affected.

but generally slow spread of this disease has given time for a comprehensive research program to develop in several states and in two regional experiment stations of the United States Forest Service. Research findings and their application in well-managed control programs may make it possible to prevent disastrous losses if control measures are wisely chosen, if control programs are adequately supported, and if such programs are continued without interruption while the threat of oak wilt exists.

History of Our Early Knowledge of Oak Wilt

In the historical section that follows, an attempt is made to list or summarize the early discoveries concerning the nature, cause and spread of oak wilt. Changing ideas in regard to the importance of the disease and concerning its rate of southward and eastward spread are reviewed. These shed light not only upon oak wilt but on the reactions of our citizens, the representatives of industry and of government, to the

threat of disastrous losses from a disease affecting an important forest tree. The other sections of the bulletin are designed to bring the reader up to date concerning all important phases of our knowledge of oak wilt.[4]

THE DEVELOPMENT OF INFORMATION AND EARLY ATTITUDES CONCERNING OAK WILT

The first part of this history touches only briefly upon the discovery, intensification, and apparent spread of the disease. It deals primarily with the response of concerned people to what they were learning about oak wilt. The second part emphasizes the development of our scientific knowledge concerning this disease up to the time when West Virginia began its own program of research on oak wilt.

THE RESPONSE OF CONCERNED CITIZENS TO THE THREAT OF OAK WILT

The oak wilt disease was named and characterized as a specific disease fatal to oak in a report of the Wisconsin Agricultural Experiment Station (Anonymous, 1942). Henry and Moses described it briefly before the American Phytopathological Society in 1943. The fungus causing oak wilt was described and named *Chalara quercina* by Henry in 1944. In the same year the report of a comprehensive study of the disease was published by Henry, Moses, Richards, and Riker.

It seems likely that oak wilt had caused some anxiety among foresters of the region for several years. Henry and Moses reported (1943) that oaks had been dying rapidly "during the last decade." Carter (1941) stated that oak disease losses had increased in Illinois since 1934. Tiemann, in 1927, described a disease of oak which caused symptoms very much like those of oak wilt. Drought and root rot were blamed for what seem to have been early losses caused by oak wilt (Carter, 1941). The oak disease survey conducted by Carter in Illinois in 1941 failed to point specifically to any new serious vascular wilt of oak, but in the section of his report devoted to *Armillaria* root rot he stated that, "staghead, general dieback and sudden dying" were especially serious in the northeastern part of the state. Tiemann (1954) recorded the early opinion that trees with symptoms of oak wilt in these early years were considered then to be victims of attack by *Armillaria*. Henry, Moses,

[4]One aspect of the story that might prove puzzling is the fact that since it was discovered, the fungus that causes oak wilt has been called by three different scientific names, *Chalara quercina* Henry, *Endoconidiophora fagacearum* Bretz, and now *Ceratocystis fagacearum* (Bretz) Hunt. The story of these names, how they were given and how changed, is told here. The important thing to remember in reading the bulletin is that all three are different names for the oak wilt fungus. This is more fully explained on pages 35 and 38 of this bulletin.

Richards, and Riker (1944) concluded rather conservatively, "Although considerations of the relationships among various important items in the oak disease complex need further investigation, the present study has demonstrated one cause for the dying of oaks, particularly in the red-oak group."

In 1944 oak wilt was reported in 23 southwestern counties in Wisconsin, 5 counties in Southeast Minnesota, 2 counties in Iowa and 1 in Illinois (Henry, Moses, Richards, and Riker). At that time species of the red-oak and white-oak groups were both shown to be susceptible; the white oaks were considerably more resistant than the red oaks. During the period 1944-1947 the known range of oak wilt was extended into many additional counties of Wisconsin, Minnesota, Iowa, and Illinois, and Bretz (1944) reported its discovery at St. Louis, Missouri. Dietz and Barrett (1946) reported that oak wilt had occurred throughout Iowa in 1944 and 1945. In 1948 the first state bulletin concerning oak wilt appeared. It was written by Dietz and Young of the Iowa Agricultural Experiment Station. They reported that all 11 oak species native to Iowa were susceptible to oak wilt inoculation and that nine of these had been found diseased in nature. In a further search for a resistant oak 17 species unknown in Iowa were tested by inoculation and found susceptible. They reported that in Pilot Knob State Park, where Hills yellow oak (*Quercus ellipsoidalis* J. F. Hill) predominated, 51 per cent of the oak had been killed by oak wilt.

At the end of 1949, seven years after the first published report concerned specifically with oak wilt (Anonymous, 1942), enough had been learned about the disease to indicate that its causal agent, *Chalara quercina,* was a dangerously pathogenic fungus. It successfully infected all oak species inoculated, killing members of the red-oak group rapidly and causing dieback of variable severity in white oak (*Quercus alba* L.) and other species closely related to it. Little was known concerning its means of spread, although spread-pattern studies indicated that its dissemination was of two types. One was a rather slow, but frequently efficient localized spread more properly considered as intensification. This had been attributed by Wisconsin workers to transmission through naturally occurring root grafts between nearby trees. The other was an unpredictable type of long-distance overland spread which seemed to occur less frequently. The successful control of oak wilt through extensive sanitation and tree removal seemed difficult and impracticable on a large scale. Less exacting procedures had proved ineffective in checking the disease. Some efforts had been made to evaluate the timber threatened and the extent to which the value was endangered by the disease.

By January 1, 1950, oak wilt was considered by most informed pathologists an immediate threat to the forests and woodlots of the Upper Mississippi Valley. It was known to be killing many different oak species in Missouri, Illinois, Indiana, Iowa, Wisconsin, and Minnesota (Kuntz and Riker, 1950 b). There was some question as to how long the disease had been present in these areas and whence it had come. Some professional foresters doubted that oak wilt was new, and they considered its danger neither startling nor especially serious (Tiemann, 1954). The concern of the people in the states affected had hardly been aroused beyond the point of considering oak wilt an interesting and perhaps potentially dangerous disease that might at some time have to be dealt with.

The year 1950 marked a decided change in this attitude. It was a year of spreading anxiety concerning the present and potential losses from oak wilt.

In 1950 oak wilt was reported in three other states (Ohio, Anonymous, 1950; Arkansas, Bretz and Henry, 1950; and Pennsylvania, Fergus and Morris, 1950) in addition to the five already known to harbor the disease. Its apparent southward spread into Arkansas seemed to threaten the large and economically important areas of oak forest extending south through Missouri and Arkansas where the upland oak forests merge with the delta and bottom land hardwoods, of which oak species are a major valued component. The flooring industry in the South and the makers of tight cooperage stock felt that their raw materials were in danger; the railroads using oak crossties, and other important oak-using industries joined in this very real concern. The discovery of oak wilt in Pennsylvania caused a similar apprehension among the users of Appalachian oak including the mining industry which utilized much oak as mine prop material. Oak wilt had been found during this year also in Nebraska, Ohio, and Kansas, but these discoveries remained unpublished until 1951 (Shields, 1951; Fowler, 1951). The presence of the disease in these states was considered by many to be the result of recent spread, and memories of the destruction caused by chestnut blight which had spread southwestward across Virginia at the demonstrated rate of 24 miles per summer (Gravatt and Marshall, 1926) were fresh enough to arouse apprehension in the minds of specialist and layman alike.

In 1950, Bretz and Long reported the isolation of the oak wilt fungus from naturally infected Chinese chestnut, a species extensively used in soil and wildlife conservation programs. This further focused the attention of a large segment of conservation-minded citizens upon the danger to oaks and chestnuts, both of which are important sources of mast for game. Fear was aroused that beech, another important

member of the Fagaceae, might become or indeed be already affected by this disease (Shenefelt, 1952).

Articles concerning oak wilt were published in popular, industrial, and semitechnical magazines under titles designed to awaken the public to both the real and fancied dangers. For example, one anonymous article (1951 b) appeared in a trade journal under the title "Oak Disease Spreading 50 M.P.H." The oak-using industries shared their apprehension with the ordinary citizen to the point where unexpected results followed. Owners of healthy oak timber throughout the threatend regions were easily persuaded to sell their immature oak stands cheaply before the disease should render them valueless. Early in 1950 Ibberson reassured the citizens of Pennsylvania that the disease was yet unknown there and, without minimizing the real danger posed by the disease in the middlewest, he attempted to offset the rising apprehension among Pennsylvania woodland owners. Later in the same year Fergus and Morris announced the discovery of oak wilt in Mifflin County, Pennsylvania.

In 1950 Milbrath wrote a bulletin on oak wilt for Californians and Hansbrough wrote a Tree Pest Leaflet for the New England Section of the Society of American Foresters. Rex of the New Jersey Department of Agriculture made a tour of the midwestern disease area, reporting his observations to the Eastern Plant Board (Rex, 1950).

It became apparent in 1950 that oak wilt was of national importance and in that year it became a matter of national concern. An allotment of funds made available by the United States Forest Pest Control Act, enabled the Division of Forest Pathology of the United States Department of Agriculture to begin a limited survey for oak wilt in Missouri, Arkansas, and in the western portions of Tennessee and Kentucky. Bretz and Henry reported the disease in Arkansas on the basis of this survey (1950).

More than survey work was needed, but federal research funds were still chiefly allocated elsewhere. Industries using oak took a pioneering step in uniting to furnish financial support for a research program of national scope with the ultimate hope that the disease might be controlled effectively before our forests were seriously depleted or the oaks were lost to our economy. The National Oak Wilt Research Committee was formed under the sponsorship of 10 trade associations[5] representing

[5]The National Oak Wilt Research Committee was comprised of representatives of the following trade associations: American Forest Products Industries, Inc., Appalachian Hardwood Manufacturers, Inc., Associated Cooperage Industries, Hardwood Dimension Manufacturers Asociation, National Hardwood Lumber Association, National Oak Flooring Manufacturers Association, Northern Hemlock and Hardwood Manufacturers Association, Railway Tie Association, Southern Hardwood Producers, Inc., and the Veneer Association.

industries dependent on oak timber as a raw material. The chairman was Leonard R. Steidel, Production Manager, Cooperage Division, National Distillers Products Corporation, who represented the American Forest Products Industries, Inc., and Henry H. Willins, Secretary-Treasurer of the National Oak Flooring Manufacturers Association was the secretary. The most important contribution of this Research Committee consisted of charting an intensive three-year research program, "—with the advice and assistance of outstanding plant pathologists" (Willins, 1951). This program was designed to supplement research already under way and it was expected to cost approximately $210,000. The Advisory Committee of plant pathologists was led by A. J. Riker of the University of Wisconsin and originally included Curtis May, then of the Division of Forest Pathology, United States Department of Agriculture; L. T. Tehon and H. B. Mills of the Illinois Natural History Survey Division; C. M. Tucker of the University of Missouri, and W. H. Bragonier of Iowa State University. Two other pathologists associated with the early work of the Committee were J. E. Kuntz of Wisconsin and T. W. Bretz of the United States Department of Agriculture stationed at the University of Missouri.

In 1951 pathologists in Michigan, Ohio, Pennsylvania, and West Virginia and men responsible for protection of the forests in Nebraska, Kansas, Arkansas, Indiana, Maryland, and Virginia realized that they also had to deal with oak wilt. Previous thinking in these states had been chiefly in terms of quarantines to keep the disease out, but now the prospects for control or eradication had to be considered. Since no one knew how the disease was spread overland, the formulation of an adequate control program was impossible. Survey was necessary, together with a research program designed to discover the means of overland spread and to devise effective control measures.

Responsible agencies in Michigan (Strong, 1951), and later in Maryland (Weaver and Jeffers, 1952) and Virginia, followed the lead of those in Nebraska, Kansas, Indiana, and Arkansas who had decided to do what they could toward keeping watch on the disease by survey activities. The undertaking of disease control in these states had to await the time when research could point the way toward a reasonable control measure designed to check overland spread, as well as to prevent the localized intensification of the disease through root graft transmission.

The Asheville, North Carolina, office of the Division of Forest Pathology of the United States Department of Agriculture assumed leadership in survey and in research for the Southern Appalachians where oak wilt was present and spreading within North Carolina, Tennessee, and Kentucky (Anonymous, 1951 a; Fowler, 1952 a).

The Pennsylvania Department of Agriculture, in 1951, obtained a $50,000 appropriation of which it allotted $8,800 to the Pennsylvania Agricultural Experiment Station for research (Anonymous, 1952). Survey and exploratory steps toward control were undertaken jointly by the Pennsylvania Department of Agriculture and the Pennsylvania Department of Forests and Waters. These agencies, as well as the Department of Plant Pathology and the College of Forestry at Pennsylvania State University, began to develop research programs along lines best fitted to their abilities or opportunities.

In Ohio (Ohio Forestry Association, 1951) and West Virginia the initial major support for oak wilt research came from industry. This permitted an early beginning on needed research in these states much as the National Oak Wilt Research Committee had earlier made possible the expansion of research in the Middlewest.

The presence of the disease in West Virginia was not confirmed until early in July 1951, when the oak wilt fungus was isolated from samples collected almost simultaneously from Monongalia, Cabell, and Pendleton counties by representatives of the West Virginia University Agricultural Experiment Station, the Agricultural Extension Service, the West Virginia Conservation Commission and the White Pine Blister Rust Control Agency of the United States Department of Agriculture (True, Craig and Barnett, 1951). All cultures were identified by H. L. Barnett, the first isolate being confirmed by T. W. Bretz of the Division of Forest Pathology of the United States Department of Agriculture.

These discoveries of oak wilt represented the first returns from an informal, state-wide sampling survey, one of several such surveys made with the encouragement and assistance of the United States Department of Agriculture's Division of Forest Pathology in southern and eastern states (Fowler, 1952 b). The West Virginia survey had been planned in Charleston, West Virginia, May 22, 1951, at a meeting with Marvin Fowler of the United States Department of Agriculture. J. G. Leach, R. P. True, and E. H. Tryon represented West Virginia University, H. L. Baker and Hays Helmick represented the West Virginia Conservation Commission, and F. W. Craig and Shirley Brown represented the West Virginia Department of Agriculture. H. D. Bennett, Forester, Appalachian Hardwood Manufacturers, Inc., represented the oak-using industries at this meeting. True and Craig were asked to coordinate the proposed survey. All interested State agencies, cooperating foresters, and many interested citizens contributed generously to its success. Governor Patteson assisted materially by making emergency funds available. Ultimately, 54 oak wilt trees were found in 1951 in 17 scattered counties

(True, Craig, Baker, and Bennett, 1951) in a sampling survey designed along lines recommended by Fowler (1951) and executed under the detailed supervision of Donald G. Lynch of the West Virginia Conservation Commission. Limited but strategic airplane scouting by Edsel France, pilot for the Conservation Commission, contributed much to this West Virginia survey. Much of the confidence with which West Virginians tackled the first oak wilt survey after the discovery of the disease here in 1951 was based on experience and understanding gained from Mr. Fowler and Dr. Bretz who together directed a training school in Missouri on oak wilt survey methods including airplane spotting of diseased trees, followed by ground checking.

It was concluded at the end of the 1951 season that oak wilt was widespread within West Virginia, that it had been in the State for several years, and that during this time it had been actively spreading, chiefly overland. The disease did not yet appear to threaten the early loss of any considerable volume of timber, and it seemed reasonable to hope that the development of a comprehensive research program might discover some practical and effective control in time to prevent disastrous loss.

On October 5, 1951, after the completion of the survey, a meeting of cooperating agencies and representatives of the State's wood-using industries was called to discuss the situation. The recommendation of the group was to postpone the undertaking of a control program until more could be learned concerning the method by which the disease was spread. A committee from the wood-using industries was formed to obtain financial support to initiate a program of research in West Virginia. Under the leadership of R. E. Johnson, Federal Coal Company, President, and of H. D. Bennett, Secretary, this West Virginia Oak Wilt Committee obtained a sum of $7,570 to inaugurate immediately a comprehensive research program by the Department of Plant Pathology, Bacteriology, and Entomology of West Virginia University.[6] Each association contributed approximately two thousand dollars. Certain of these industrial groups had previously contributed substantially to the National Oak Wilt Research Committee for the financing of oak wilt research in the middle western states. Later West Virginia was included among the research agencies supported by the National Oak Wilt Research Committee, and funds for the support of oak wilt research in West Virginia were channeled through the National Committee. The

[6]Contributing associations of West Virginia oak-using industries included: The West Virginia Forest Fire Protective Association, composed of forest land owners; The Appalachian Hardwood Manufacturers Association; The West Virginia Coal Operators Association and West Virginia Railroad Association.

National Oak Wilt Research Committee under the leadership of Leonard Steidel and later under that of Sam Nickey (with the help of the advisory committee and its chairman, A. J. Riker, of the University of Wisconsin), continued to underwrite research in West Virginia and the Midwest for the three-year term initially proposed. During recent years oak wilt research in West Virginia has been financed by state and federal appropriations.

EARLY RESEARCH ON OAK WILT

The first reports and earliest studies of oak wilt and of the fungus causing it (Anonymous, 1942; Henry and Moses, 1943; Henry, 1944; Henry, Moses, Richards, and Riker, 1944) were supplemented in 1947 by an additional publication by Henry and Riker dealing primarily with the effectiveness of different infection courts and the importance of wounds for artificial infection. By 1947 oak wilt had been distinguished from the complex of unknown and often minor diseases (Carter, 1941) affecting oak and established by state and federal workers in Wisconsin as the major disease affecting oaks in that state and the upper Mississippi Valley.

The activity of this hitherto unknown fungus within the vessels of the outermost annual rings of the sapwood caused leaves of affected trees to droop, sometimes to wilt, and in many cases to fall from the tree, usually after their tips and margins had turned bronze or brown. The disease, therefore, belonged in the category of the vascular wilts, taking its place beside the Dutch elm disease, persimmon wilt, mimosa wilt and the tree wilts caused by *Verticillium*.

The fungus causing oak wilt was named *Chalara quercina* by Henry (1944). He described the characteristics of the vegetative mycelium of this fungus as it developed on different nutrient media and the endoconidial type of asexual spores produced by this mycelium on these media or when grown aseptically on bark and wood. Henry reported the known host range to include white oak, red oak, scarlet oak (*Q. coccinea* Muenchh.), bur oak (*Q. macrocarpa* Michx.) and black oak. The oak wilt fungus had been isolated from samples taken in Illinois, Iowa, Minnesota, and Wisconsin during 1941 and 1942.

Henry, Moses, Richards, and Riker (1944) described the disease itself, its symptoms, its distribution within affected trees, its manner of spread, its severity on a wide variety of sites, and its importance as a tree disease affecting forests and woodlots.

Symptoms. Trees of all sizes became diseased. Symptoms appeared from late May to late September, but most trees were affected and symptoms were most conspicuous during June and July. The nature and

extent of the symptoms in affected trees differed considerably between species of the red- and the white-oak groups.

Among species of the red-oak group, symptoms appeared first near the top of the tree or the tips of the branches. Succulent, immature leaves blackened progressively from the tip toward the leaf base, they curled, drooped, and remained attached after wilting. The succulent young leaves of sucker shoots, sometimes produced after initial defoliation, also showed similar symptoms. Affected mature leaves of trees initially showing symptoms after the beginning of June were at first pale and a little crinkled. They became bronze or brown from the tip toward the leaf base and the part of the blade nearest the petiole was the last to become discolored. Mature leaves did not wilt but remained quite turgid, falling from the tree at all stages of symptom development and in widely varying numbers. Frequently, but not always, the outer one or two annual rings of the sapwood became partially or completely discolored. When present, these black or brown internal discolorations aided in the diagnosis of diseased trees. The leaves, twigs, branches, trunk and roots of diseased trees died in the order given. The annual radial increments of diseased trees usually indicated normal growth up to the year in which wilting occurred. Trees of the red-oak group were reported to die usually in the year they first showed symptoms, but in the case of large trees, or those affected late in the season, death was sometimes delayed until the following year. No diseased tree of the red-oak group was known to have recovered.

Fewer trees became diseased, and symptoms were usually less extensive, among members of the white-oak group. Mature leaves of white oaks and their close relatives became discolored basipetally as in the case of red oaks. Instead of becoming bronze at first, affected leaves initially took on an abnormal dark green color with water-soaking of the tissues. Later a bronzing was sometimes evident but most frequently a brown or light tan discoloration typified diseased white oak leaves. Leaf fall from affected trees was less in the case of white oaks than in red oaks, and the extent of symptoms was frequently limited at first to one or several groups of twigs or branches. Later a progressive dieback marked the spread of the disease through the crown. Internal vascular discoloration was found consistently associated with the disease in trees of the white-oak group.

Distribution of the Oak Wilt Fungus Within Diseased Trees. *Chalara quercina* was consistently isolated (Henry, Moses, Richards, and Riker, 1944) from living diseased twigs, branches, trunks and roots. When attempts were made to isolate the fungus from dead wood,

Dothiorella quercina (Cke. and Ell.) Sacc. and other secondary fungi usually overran the culture plates. A few of the isolations attempted from leaves proved successful. By standard procedures *C. quercina* was isolated from 104 of 108 wilting red oaks sampled in 1941 and 1942 and from 12 of the 14 white oaks sampled. Fifty-six diseased oaks affected by die-back, staghead, twigblight, cankers and other non-wilt symptoms failed to yield *C. quercina* when cultured.

Healthy black oak sprouts 1½ to 5 inches d.b.h. growing in a disease-free woodland near Madison were inoculated chiefly in chisel wounds into the two outermost annual rings of the main trunk. *C. quercina* was successfully reisolated from all inoculated trees which developed symptoms. Uninoculated controls and inoculated trees which showed no symptoms never yielded *C. quercina.*

Nineteen of 37 black oaks inoculated in August 1941 showed symptoms before the end of September. Nine more showed symptoms before the leaves matured the following May. In June 1942, three additional trees died after leafing out normally. Six other trees failed to develop symptoms.

Two white oaks inoculated in 1941 exhibited symptoms early, but showed signs of recovery in 1941. In 1942 only slight die-back and no wilt symptoms could be noted in them.

The Pattern of Disease Spread. In areas where the disease was present (Henry, Moses, Richards, and Riker, 1944) its pattern of spread was found to be very erratic, but usually newly diseased trees were found in close proximity to others previously diseased. Losses resulting from this local spread were occasionally very great. If one member of a sprout group became infected, the sister sprouts usually showed symptoms the same or the following season, and *C. quercina* could be isolated from the tissues uniting the sprouts at their base. It was further pointed out that oak roots from nearby individual trees sometimes anastomose, becoming organically united under ground, as Weir had shown in his studies of *Endothia gyrosa* (1925). Henry, Moses, Richards, and Riker (1944) concluded that the localized spread of this vascular wilt might occur through such previously existing anastomoses or root grafts. Varrall and Graham (1935) had shown earlier that the Dutch elm disease, another vascular wilt, was occasionally transmitted in this manner.

The separation of such local areas of disease from one another by distances of several hundred yards or more, and the fact that occasionally trees became infected in areas previously free from disease, indicated that other means of spread existed.

Lack of Relationship of Site and Soils to Disease Intensification. In an attempt to explain the rather erratic differences in rate of intensification and spread, an effort was made by Henry, Moses, Richards, and Riker (1944) to correlate incidence and severity of the disease with specific site factors. No relation was found with soil characteristics, the nutrients they contained, or with soil acidity. Trees became diseased near swampy land, on hillsides and plateaus, in open fields and in closed forest stands. Trees were found affected by oak wilt on pastured or unpastured land of good and inferior quality as expressed in terms of tree growth, so it was concluded that these factors did not appreciably affect the incidence or severity of the disease. The puzzle remained as to why the rate of intensification and spread was so variable.

Early Estimates of the Economic Importance of Oak Wilt. Henry, Moses, Richards, and Riker (1944) attempted to weigh the importance of oak wilt as a threat to forests and woodlots. They pointed out that oaks belonging to the red-oak group were severely affected and that white oak and the closely related bur oak seemed relatively tolerant. Oaks reportedly made up 80 per cent of the trees in the affected woodlands of Wisconsin. The total income from Wisconsin farm woodlands had been reported in 1929 at $17 million and at $10 million in 1939. In 1937, $5½ million of this income was reported to have been derived from oak. The following facts were pointed out for consideration in evaluating the importance of oak wilt losses in forests and wood lots: (1) Salvage operations on dead, immature trees cannot realize the full value of the timber; (2) After such oaks are killed, a number of years will be required before the area can be stocked with timber of comparable value; (3) Much of the natural reproduction would normally be expected to be of sprout origin, but since the disease prevents the successful sprouting of infected stumps, the return to adequate stand density in these areas would be further delayed or perhaps prevented; and (4) The loss of oak might be irreparable in areas where oak species are unqualifiedly the best trees for the site. The substantial but intangible value of oaks as street, lawn and park trees was not included.

Effect of Place of Inoculation Upon the Incidence and Pattern of Symptom Development. Henry and Riker (1947) carried on further inoculation studies from which they concluded that no tree parts became infected without wounding, but that wounds penetrating to the xylem of roots, stems, branches or twigs usually made suitable infection courts. Root and stem wounds permitted infection more frequently than did wounds in petioles or leaves. Wounded and non-wounded roots always failed to serve as effective infection courts unless the inoculum was applied directly to the wound. Black oaks inoculated in the roots or

trunk first showed symptoms in scattered branches of the upper crown, but if inoculated in leaves, twigs and small branches they first showed symptoms at or near the point of inoculation. Symptoms soon spread to involve the whole crown in either case.

A study of the distribution of the pathogen in naturally infected red and black oaks showed the fungus to be present in the branches of all trees tested, in the twigs and trunks of nearly all, and in the roots of more than half of the 26 root systems studied. No differences seemed to exist between the distribution of the fungus in red and black oaks.

Roots were found to harbor *C. quercina* over winter under natural conditions and the fungus was occasionally isolated from leaves and the petioles of fallen leaves.

The distribution of the parasite was found to be nearly complete throughout the above-ground parts of 16 stem-inoculated black oaks when symptoms first appeared in any part of their crowns. The parasite was isolated from the two outermost rings of sapwood even in naturally infected trees showing initial symptoms.

Early Suggestions in Regard to Disease Control. Conclusions relating to possible control of the disease were drawn by Henry and Riker from their studies (1947). Members of the white-oak group, themselves less seriously or conspicuously affected than the red oaks, might serve as inconspicuous sources of inoculum. The roots of red and black oaks harbored the fungus over winter, making it appear likely that root-graft spread occurred frequently in nature. Detached leaves likewise harbored *C. quercina* for a time, and could serve as vehicles of local spread. The presence of wounds appeared potentially important in possible spread by insects, birds, and rodents. Tools were suspected as both wounding and disseminating agents. Until the pathogen could be found fruiting on the surface of affected trees, the chance seemed slight that rain or surface water would spread the disease. The extent of vascular infection beyond the localized areas of early symptoms suggested that pruning out the first symptom branches of red and black oaks would not rid the trees of the disease. Since vigorous trees became diseased in nature, fertilization or irrigation seemed unlikely to protect trees or to enable them to recover. Sanitation measures which would include the killing or removal of diseased roots were suggested and attention was called to preliminary encouraging results obtained by Dietz and Barrett (1946). In commenting in 1948 on possible control measures, Riker (1948) told the Midwestern Chapter of the National Shade Tree Conference, "Someone is sure to ask 'What can we do about it?' We are frank to say 'We do not know.' If you want to try something on an

experimental basis you might; remove any affected trees, keep a close watch on others near them, remove any newly infected trees *within a week* of the first symptoms."

The First State Oak Wilt Bulletin. In 1948 the first state bulletin concerning oak wilt appeared. It was written by Dietz and Young (1948) and entitled *Oak Wilt, A Serious Disease in Iowa*. To the symptoms already reported, the authors added the curling or slight upward cupping of leaves, before and after discoloration. They also reported that red oaks infected early in the season usually died in one or two months. Fall-infected trees died the following spring, after retarded and limited foliation. In bur oaks and white oaks infection might persist for years, a few branches dying annually. They reported the known host range to include the 11 oak species native to Iowa. Nine of these were reported to have become infected in nature.[7] In a further search for a resistant oak 17 species unknown in Iowa were tested by inoculation and found susceptible.[8]

In an attempt to evaluate the economic importance of the disease to Iowa, data had been taken by Dietz and Young (1948) on 45 wood lots of northeastern Iowa, the combined area totalling 1,933 acres. Trees of the red-oak group made up 51 per cent of the total trees and contributed 59 per cent of the total volume. In one 40-acre wood lot the volume of timber lost due to oak wilt constituted 69 per cent of the potential annual growth of all trees. Farm wood lots near Dubuque had lost 25 per cent of their oak population. In Pilot Knob State Park where Hill's yellow oak predominated, 51 per cent of the oak had been killed by wilt.

Dietz and Young (1948) stated that no satisfactory control method had been found, but their experimental work with sanitation and other suppression measures, begun in 1943 in Dolliver and Pilot Knob State Parks, had given some encouraging results. At two different isolated centers of infection the infected trees were removed, and all red oaks within 50 to 100 feet cut down. The logs were hauled away from the site and all broken oak branches, brush and chips were burned. During the three years that had elapsed since the treatment no new oak wilt had been found in these plots. The removal of the dead and diseased oaks alone had also stopped or retarded disease spread. Pruning out diseased branches of white oaks 3-4 feet beyond the extent of the symptoms sometimes prevented subsequent spread of the disease within individual white oak trees.

[7]*Quercus alba, Q. borealis, Q. ellipsoidalis, Q. imbricaria, Q. macrocarpa, Q. marylandica, Q. muelenbergii, Q. palustris,* and *Q. velutina.*

[8]*Quercus coccinea, Q. falcata, Q. falcata* var *pagodaefolia, Q. gambelii, Q. garryana, Q. hemisphaerica, Q. laevis, Q. laurifolia, Q. montana, Q. nigra, Q. phellos, Q. prinus, Q. robor, Q. shumardi* var. *texana, Q. suber,* and *Q. virginiana* var. *maritima.*

Tentative control recommendations were offered in this bulletin by Dietz and Young: (1) Prompt removal of diseased trees felled carefully to avoid injury to others nearby, "—and thus transmitting the disease"; (2) the stumps of diseased trees should be peeled and the bark, small branches, and the leaves piled and burned on the peeled stump; (3) Slabs of logs removed for utilization should also be burned; (4) Painting the peeled stump with creosote was suggested for times when burning was unsafe; (5) Pruning out the disease from white oaks of high individual value seemed possible.

The bulletin concluded with a brief description of pathologic conditions which may be confused with oak wilt. Frost injury, anthracnose, girdling of branches by squirrels and deer mice, effects of the oak twig girdler, and cicada injury were included.

Iowa Research on Spread of Oak Wilt in the Forest and Within the Tree. In 1949, R. A. Young, then of the Iowa Agricultural Experiment Station, gave an account of detailed research findings which supplemented the practical information contained in the Iowa bulletin. Young determined characteristic patterns of disease spread, based upon data from mapped study plots. Although not all susceptible oaks close to previously infected trees became diseased during the period of study, the average distance of spread from diseased to healthy trees approximated 30 to 40 feet and usually no infection was observed beyond those diseased trees closely surrounding the first point of infection. These spread-plot data were considered inconsistent with what would be expected if the disease were disseminated by wind-borne spores or by insects. He referred to the previous work of Wilson and Baker (1946), and Woftenbarger and Jones (1943), who had shown that air-borne and insect-borne diseases had a new infection incidence rate that varied inversely with the square of the distance from the source.

Young's studies (1949) indicated that incidence of infection was not clearly related to size of tree. They showed, too, that in northern Iowa the white oaks were affected by the wilt almost as often as were red oaks. Many red oaks, however, were found killed at Pilot Knob State Park where many white oaks showed only a few dead branches. Studies of symptom incidence indicated that initial symptoms usually appeared in June or July, but in 1947 June was cool and rainy, and symptoms that year appeared chiefly in July.

Young (1949) made a detailed investigation of fungus distribution within the host, reaching conclusions similar to those of Henry and Riker (1947). His studies, however, included a histological investigation of the infected sapwood vessels. Young observed mycelium in the vessels

of the midribs of leaves, in the xylem of petioles as well as within the outer annual rings of twigs. The mycelium generally grew longitudinally within infected vessels. Mycelial development was often sparse, and numerous condia were also found within the vessels. The presence of the fungus in leaves and twigs indicated to Young that symptoms need not be considered due to the action of a toxic substance acting at a distance, but suggested that mechanical plugging of the vessels with mycelium should not be considered the sole cause of wilting. The small conidia themselves could be subject to rapid distribution in the transpiration stream.

"In tests in which spore suspensions were forced through 12-in. sections of red, white and bur oak twigs under pressure, spores were present in the first drops of liquid to be drawn from the twigs. Numerous chains of 8 to 10 spores passed through the vessels intact."

Young's extensive physiological studies (1949) included some which led him to consider that toxic substances produced by the fungus could be responsible for the symptoms shown by diseased trees. He placed red oak cuttings into dilutions made from used liquid media which had been freed of the fungus by filtration, and found the oak cuttings severely wilted after 24 hours. After 70 hours, large necrotic areas developed between the veins of wilting leaves. After 120 hours, leaves were dry and papery and showed discoloration patterns like those on trees inoculated in the greenhouse. Tomato cuttings responded in a similar manner. When tomato cuttings which had wilted after 48 hours in the filtrate were cut off some distance above the original absorbing cut and placed in distilled water, they failed to recover. A simple plugging at the original cut surface seemed not to be solely responsible for the wilt.

The Sexual Stage of the Oak Wilt Fungus Found, and the Fungus Renamed.[9] A very significant contribution was made to our knowledge of the oak wilt fungus itself during 1951. Bretz (1951 b) obtained the perithecial stage by culturing individual isolates together in pairs. Perithecia were produced only when certain isolates were paired with certain others. The ascospores were unicellular, hyaline, elliptical, curved on one face somewhat like the segments of an orange. No perithecia found in nature were reported in this paper. No mature asci were seen and it was assumed that ascospores were liberated at maturity by the dissolving of an evanescent ascus wall. Perithecia were minute, black and flask-shaped, and the necks were about the same length as the width of the swollen bases. These ascocarps grew singly,

[9]An explanation of the naming and renaming of the oak wilt fungus is given on pages 35 and 38.

with little more than the necks protruding above the level of the subiculum. These necks terminated above in a cluster or fringe of colorless hyphae. The white globular spore masses extruded from the ostioles made the perithecia visible to the unaided eye. The ascospores were easily distinguished from the cylindrical endoconidia of the asexual stage.

This same year, Hepting, Toole, and Boyce (1951) reported the culturing of isolates of *C. quercina* from 28 separate areas and stated that when certain of these were paired with, or spermatized by endoconidia from certain others, perithecia were produced in culture. Thus, the fungus was heterothallic. They reported also that one isolate produced fertile perithecia without spermatization or pairing. Later work was to determine by the reaction of single-spore cultures from this isolate that it consisted of a mixture of two compatibility types, each hermaphroditic, self-sterile, but cross-fertile (Hepting, Toole, and Boyce, 1952 a, 1952 b). The authors pointed out that since the ascospores are produced in a sticky mucoid matrix nearly insoluble in water, they are presumably better adapted to long-distance overland spread than the comparatively unprotected endoconidia.

STUDIES RELATED TO THE SPREAD AND CONTROL OF OAK WILT, 1950-51

Kuntz and Riker (1950 a) reported that attempts to control local spread of the disease by the eradication of dead and wilted trees or by the periodic application of DDT sprays, or both combined, and by disposing of the diseased material in a variety of ways had all failed to control the local spread of oak wilt. Poisoning the roots of healthy oaks adjacent to currently diseased trees had, however, given control of local spread, presumably by interrupting the network of grafted roots which they often found uniting the root systems of black oaks. Dyes and the spores of *C. quercina* placed near such grafts on the side adjacent to the diseased trees passed across the graft into the root system of the adjacent healthy trees where the dyes were easily visible and from which *C. quercina* was readily isolated. Kuntz reported later (1950), "Pairs of oaks have been infected by artificially inoculating a root of one tree grafted to that of the second." Poisons moved through such grafts and into the healthy root systems for demonstrated distances up to 28 feet from the diseased tree. The physical interruption or chemical poisoning of the network of living grafted roots might be expected to check root-graft spread.

Beckman and Kuntz (1951) showed that poisons, dyes, and radioactive iodine passed readily from tree to tree through natural pre-existing root grafts. A single jack oak (*Q. ellipsoidalis*) poisoned with sodium

arsenite passed on enough of the poison through root grafts to kill five nearby trees. When these five were given comparable treatment, 21 additional nearby trees died. The treatment of these 21 resulted in the death of 10 additional trees. Thus it was concluded that at least 36 trees in this stand were united directly or indirectly to the root system of the first tree poisoned. Similar situations were demonstrated by the use of radioactive iodine. This substance went 12 feet upward in treated trees during the first 15 minutes after its introduction into the tree. Its downward progress was considerably slower except when root grafts were present.

Kuntz and Riker (1951) reported that two years after removal of 18 single diseased oaks at the time of wilting, four of the 18 infection centers continued free from disease. In 11 other similar plots the spread of the disease seemed to have been retarded by the removal of the diseased trees. In 12 similar single-tree sites where the initial oak wilt tree had been poisoned, no new cases of disease had developed (6 of the plots were 4 years old). In 10 other such plots trenching between wilting and healthy trees had prevented disease spread for two years. The authors reported that the Wisconsin Conservation Department "has developed a tractor-drawn knife that moves 100 feet per minute and cuts roots to a depth of 36 inches." Wysong, in charge of the Cook County Illinois Forest Preserve, modified a mechanical ditch-digging tool which dug a ditch and filled in the loosened earth, stones and debris behind itself. Neither of these mechanical devices could be widely used in the rough topography and the shallow and often rocky forest soils of West Virginia.

Riker (1951), in a report on the natural spread of oak wilt in local areas where no control had been attempted, stated that 41 such plots had been established and mapped between 1939 and 1949. Usually a single dominant tree was the first to be affected at any site. Radial spread of the disease outward from the initial tree usually occurred at a rate of one or two new trees each year. In only 3 of the 41 mapped plots had the spread of the disease appeared to stop naturally.

Hoffman (1951), at Iowa State College, attempted to approach the problem of oak wilt control from the angle of chemotherapy. He screened more than 80 chemicals for their suitability as chemotherapeutants, testing them for: (1) their ability to inhibit spore germination at four different concentrations, (2) their ability to "neutralize" the effects upon tomato cuttings of the "toxins" produced by the pathogen in artificial liquid media, and (3) the degree of injury caused by the chemical alone to normal red oak cuttings.

Hoffman itemized the characteristics desired of a chemotherapeutant and reported that 15 of the chemicals tested, representing a wide range of organic groupings, possessed a high degree of fungitoxicity, a low order of toxicity to the host, and had shown a capacity to neutralize the toxin produced by the pathogen. He concluded, "The fact that a wide variety of chemicals neutralize the toxin suggests a mechanism general in action rather than specific."

In 1951, Fowler gave a comprehensive review of currently known information about oak wilt. He included the findings of the Division of Forest Pathology concerning oak wilt distribution obtained in a series of surveys; perhaps the greatest contribution was a detailed description of survey procedures that had proved useful in the course of air and ground survey work pioneered in by the U. S. Department of Agriculture in the summer of 1950.

Fowler reported that preliminary use of airplanes to scan for the discolored tops of trees affected with oak wilt had shown that diseased oaks even in the understory of a forest could be readily distinguished, often even at considerable distances. Early flights in Missouri, Arkansas and parts of western Kentucky and Tennessee were made at heights of 100 to 200 feet above the treetops following flight lines set approximately 50 miles apart in a small-percentage sampling survey of the area. Generally speaking the airplanes scouted wooded areas readily accessible from passable roads to make the follow-up work on the ground easier. He pointed out subsequently (1952 b) that three men would require 320 days on the ground to cover the area scouted by the plane crew in a single day.

In air surveys conducted during most of the summer season, current-season oak wilt trees could be readily distinguished from healthy trees or trees that were completely dead. A few diseased trees which had undergone severe defoliation became difficult to distinguish late in the season. It was more difficult to distinguish oak wilt trees from trees dying from lightning strike, or from the browning crowns of trees purposely poisoned or girdled. Usually in these cases, however, the crown discoloration was more uniformly brown and little leaf fall occurred until the end of the summer. Frequently in circling suspicious-looking trees it was possible to see the scar inflicted by lightning. The partially defoliated crowns of oak wilt trees appeared thin by comparison. Some of their leaves were usually partly green for some time after the initial onset of symptoms, so that the crowns of trees suffering from oak wilt were either greener or less clearly brown than was the case for trees dying from the other causes mentioned. When a suspicious-

looking tree or group of trees was observed from the air, the observer marked the spot as accurately as possible on a map and related it to such landmarks as might be visible and helpful to the ground crew in finding the tree.

The use of low-speed high-winged aircraft in which the observer would have a good view of the forest below was recommended. Helicopters were quite suitable but their cost of operation was much higher. A light plane with a 300-h.p. engine and a variable-pitch propeller which allowed for rapid climb at a steep angle proved to be the best of the planes tested.

Fowler recommended that every state in which oaks are of commercial importance should make at least a sampling for the oak wilt disease. He pointed out that low-flying planes following flight lines 10 to 50 miles apart could be expected to determine whether oak wilt was well established within a state. One observer in a light plane could scout 17-18 square miles of forest land per hour under ordinary conditions. With two observers this area could be doubled. This conclusion was based on the assumption of an air speed of 80 miles per hour and that each observer could survey a strip of forest 1/4 mile wide on his side of the plane. This estimate included a deduction of 10 to 12 per cent to compensate for circling required to permit the careful examination of suspicious-looking trees and to enable the observer to mark the location of the tree.

The Origin, Spread, and Present Known Distribution of Oak Wilt

When oak wilt was first described, in 1942, it was thought to be confined to the upper Mississippi Valley. In 1950 it was reported in Pennsylvania. In 1951 it was discovered in West Virginia and in neighboring states to the east and south. It is not correct, however, to conclude that the disease spread eastward as fast as the successive reports of its discovery in different eastern states suggested. Oak wilt had already been present in West Virginia at two widely separated locations for at least five to ten years before it was recognized and identified in 1951 (True, Craig and Barnett, 1951). Recent studies in North Carolina and eastern Tennessee (Boyce, 1957) indicate that the active spread of oak wilt there has been from centers of comparatively recent origin. The individual infection centers first found in Pennsylvania included some which were larger (Anonymous, 1952) and seemed older than those found in other eastern states. Variations in the intensity of factors that favor local spread (Gillespie and True, 1959) might lead to erroneous conclusions regarding the relative ages of infection centers, however, if such conclusions were based solely on size comparisons.

Oak wilt has been found in additional counties of all of the 18 states in which the disease was known in 1951, and it has recently been found in Oklahoma (Verrall, Toole and Lightle, 1959). Bretz and Jones (1958) reported the known distribution of oak wilt in the United States at the end of 1957. In Figure 2 (map of U. S. attached inside back cover) this information is so shown as to indicate also: (1) counties known to have oak wilt trees prior to 1950, (2) counties first found to contain diseased trees during 1950 and 1951, and (3) counties in which the disease was discovered after 1951. It seems reasonable to conclude either that the disease is now spreading quite slowly overland in spite of seemingly variable rates of local intensification, or that oak wilt may now be at the limits of its potential distribution because of factors so far undetermined or poorly understood.

The sensitivity of the oak wilt fungus to high temperatures (Bretz and Morison, 1953) might be expected to set a southern limit to its spread. This high-temperature sensitivity might conceivably tend to limit overland spread under conditions that would still permit root-graft transmission to continue. In warmer regions, or in areas affected by high summer temperatures, therefore, overland spread might occur chiefly during a limited period in the spring or during favorably cool years, while root-graft spread resulting in local intensification could be expected to continue during the intervening periods of higher temperatures. There is no indication that this is the situation in West Virginia where long-distance spread has seemed to be more important than local intensification except, perhaps, in some mountainous northeastern counties, where shallow soils seem to favor local spread (Gillespie and True, 1959). A comparative scarcity of oak westward and perhaps northwestward might be considered a factor checking dissemination in these directions. Toward the eastern limits of its known distribution there seems to be no explanation why oak wilt should not have spread in recent years from known infested counties of Pennsylvania northward, or why it has not been found east of the Susquehanna River. Recently state-wide surveys have been made annually in Pennsylvania. Such surveys would be expected to reveal new infection centers if present beyond the currently recognized limits of this disease area. A comparable situation exists in Virginia where the disease is known on western mountain ridges, but has not been found in comparable forested land to the eastward.

Consideration of the apparently recent eastward spread of oak wilt (Fowler, 1952 b) and of its seeming failure to continue that spread further east, has sharpened the interest of investigators with regard to

the possible origin of the oak wilt disease. It was suggested early that the oak wilt fungus might be of foreign origin (Orton and Gravatt, 1953), introduced into the midwestern United States early in the twentieth century. The fact that Chinese chestnut (*Castanea mollissima* Bl.) is affected by the oak wilt disease has suggested to some that the oak wilt fungus, *Ceratocystis fagacearum*, like *Endothia parasitica*, the cause of chestnut blight, is of oriental origin. The extreme susceptibility of Chinese chestnut seems at least to make it unlikely that *C. mollissima* served as a host in its native region. Also, the fact that foreign species of oak so far tested have proved susceptible (Bretz, 1952 b, 1955, 1957; Hoffman, 1954), and the comparatively resistant nature of our native white oaks (Parmeter, Kuntz and Riker, 1956; Jones, 1958) do not substantiate or encourage the concept that oak wilt is of foreign origin.

A second concept regarding the possible origin of oak wilt is that the fungus, as we know it today, is a mutant from a pre-existing saprophytic or weakly parasitic form, or that by mutation its potentiality for effective spread has been increased. The fungus has already been shown capable of natural mutation under forest conditions (Barnett, True and Brown, 1954). It is conceivable that mutations favoring its development as a dangerous parasite could have occurred.

The disease may have spread slowly from the time and place of its origin until it reached an area where the presence of one or more new vectors increased the speed and efficiency of its dissemination. The danger that this can happen today, or in future years, will persist to threaten us so long as the disease continues to enter new territory or to contact new potential vectors. Jewell (1956) and Dorsey and Leach (1956) pointed out a mutualistic relationship between the nitidulid beetles and several associated fungi, among which the oak wilt fungus may have recently become included. If unchecked, the extent to which *C. fagacearum* will be involved in this association with potential vector insects may be expected to increase. This can result in a corresponding increase in the rate of disease spread in areas where control measures are not undertaken and continued.

Hosts capable of harboring the oak wilt fungus without showing conspicuous symptoms of the disease (members of the white-oak group) are found wherever oak wilt is severe on the more susceptible species. This may suggest that the white oaks were and will continue to be a source and a reservoir of infection.

A radically different concept is that the disease as we know it today is indigenous to the entire area which it now occupies and that it has long existed unrecognized over all this area. Recent concern, interest,

and intensive study might have revealed its previously unrecognized presence and damaging nature.

The authors are inclined to believe that oak wilt is of middle western origin and that it spread chiefly eastward partly for reasons already mentioned (Fowler, 1952 b) and partly because of the westerly winds which blow over the middlewestern disease area during the spring and early summer months (Thompson, 1951). Such winds could help to move contaminated members of vector insect populations gradually eastward. The failure of the disease to continue its eastward progress seems likely to be more apparent than real. It is possible that unrecognized conditions at the edge of the currently known disease area may bound this margin with unrecognized natural barriers, but this seems unlikely. It is also possible that the control programs in the eastern states are exerting some check on disease spread.

Regarding the possible antecedent form of the fungus or of the disease from which the present form may have arisen, we can only speculate. It is reported, however, (Buchanan, 1957) that in Missouri pressure cushions (see page 40) seldom develop to crack the bark of dying diseased trees. The subcortical fungus mats are thus not often exposed to infestation by potential insect vectors. It is conceivable that the disease became potentially more dangerous and capable of extensive overland spread when a fungus mutation occurred that favored the development of these pressure cushions or allowed them to develop more frequently.

It does not seem probable that the oak wilt disease, as we recognize it today, is indigenous to West Virginia. If this were the case, we should expect to find a greater number of large areas of dead and dying oaks (Gillespie and Craig, 1958) and more oak-free areas scattered through our natural stands. White oaks too, would be expected to be diseased more often than they are, and these resistant species might be expected to make up a larger proportion of our older mixed stands.

The spread of oak wilt through West Virginia probably occurred as part of a slow eastward extension of the infection area. With this in mind, let us examine the distribution of known separate infection centers in Pennsylvania, Maryland, Virginia, and West Virginia. The relationship of disease incidence to the nature of the terrain, to the soil characteristics, and to land use in and near the Appalachian mountains may well be considered in examining Figure 3. It is clear that in going from west to east across that part of the Appalachians situated near the southern border of Pennsylvania, in Maryland, in the northeastern counties of West Virginia and in the western mountainous counties of

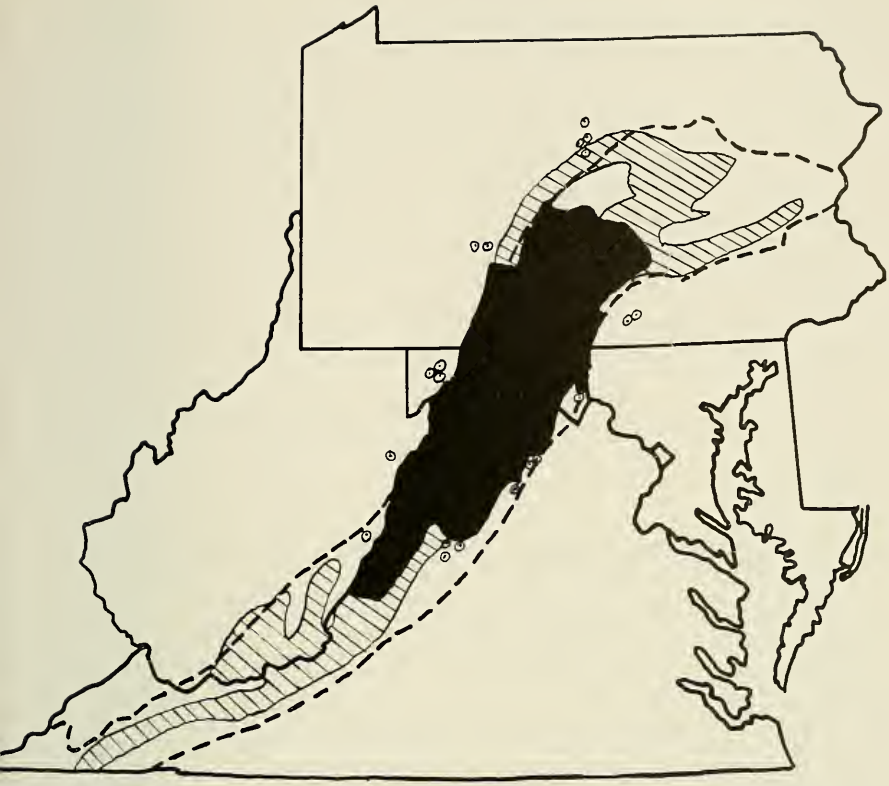


FIGURE 3. The location of known area of oak wilt concentration in and near the Appalachian region of Pennsylvania, Maryland, Virginia, and West Virginia, and in relation to the shallow soils of the Muskingum-Lehew Series. Note that the coincidence of area of disease concentration (solid black) with this physiographic area (within the dash lines) and these soils (diagonal lines) is almost exact in the middle range of their distribution but that the disease has not yet spread into their northern and southern extermities. Physiographic and soils information taken respectively from U.S.D.A. Soil Conservation Service map entitled *Problem Areas in Soil Conservation* dated May, 1950, and from USDA yearbook for 1938 entitled *Soils and Men*.

Virginia, the incidence of the disease is markedly greater within the mountainous regions. This may be due in part to the greater extent of nearly continuous forest land comprised of stands made up chiefly or importantly of oak species (except for limited areas where northern hardwoods occupy the colder sites). The Appalachian region also includes within it large areas of shallow soils which are here associated with local intensification of the disease (Gillespie and True, 1959).

If we consider the north-south axis of the Appalachians, we find the same conditions favorable to the spread of oak wilt throughout, but the distribution of the disease along this favorable axis is by no means uniform nor coextensive with the Appalachian soils and terrain. This suggests that oak wilt has not yet had time to spread uniformly within, nor to extend its range throughout, the Appalachian region. So far, it seems not to have spread into the northern end of that region where it extends into Snyder, Union, Northumberland, Montour, and Columbia counties in central Pennsylvania, where no cases of oak wilt had been found through 1957. Time, then, seems to have been a limiting factor since the known distribution of the disease is not what we should expect if it had been in the Appalachians for an indefinitely long period.

It seems likely that oak wilt, unless checked by control measures, will continue to spread eastward and northward from its present known boundaries in the states of Pennsylvania, Maryland, Virginia, and North Carolina.

The Economic Importance of Oak Wilt in West Virginia

The economic importance of a disease is based upon the economic value of the crop threatened and the degree to which the crop is damaged by it. At present the value of the oak timber crop seems the more easily estimated, but the threat of ultimate disastrous loss also seems very real unless oak wilt can be controlled effectively.

The economic importance of oak to West Virginia is emphasized by the fact that more than 75 per cent of the land surface is too steep to cultivate and too subject to erosion to utilize safely or profitably for pasture, as indicated in Table 1. Forests will always constitute a resource of primary importance to the State, and the health of these forests will continue to be of legitimate concern to the citizens of West Virginia.

Table 1. Current and Appropriate Acreages of Cropland, Pasture, and Woodland for West Virginia

Situation	Cropland		Pasture		Woodland		Total*	
	Acres	%	Acres	%	Acres	%	Acres	%
Current	1,740,000	12.2	2,603,000	18.3	9,907,000	69.5	14,250,000	100
Appropriate**	1.530,000	11 8	1,647,000	11.6	10,922,000	76.6		

*Excludes from consideration National Forest acreage, and 350,000 acres of idle land of which 71,000 acres are suitable for woodland.

**Based on unpublished data of the Soil Conservation Service (1956).

The early establishment of the Monongahela National Forest with flood control chiefly in mind emphasized and justified the earnest desire of West Virginians to keep the soil surface of their watersheds and rough lands well covered with forests.

Surveys in 1949 showed that forests covered nearly 70 per cent of the land area of the State (Wray, 1952). The major forest types on the 9,859,400 acres of commercial forest land are listed in Table 2.

The oak types are the most extensive; the red-oak type alone makes up nearly one-third of the forested area, occupying a wide variety of sites. The chestnut-oak type, characteristic of the high rocky ridges, and the white-oak type, on the richer well-drained soils, each covers 9 per cent of the forest land. From the fact that the three oak types alone cover one-half of the commercial forest land of the State, it is evident that oaks are of major importance in West Virginia.

If oaks should gradually disappear from our hillsides, their place would be taken on most sites by trees of considerably inferior quality. On some sites they might be replaced only by woody shrubs. Such ground cover would be a poor substitute for the oak when considered as a source of logs or lumber, as game food, or as watershed protection.

Our West Virginia forests have been important in attracting tourists, hunters, and fishermen. Visitor-related industry is now said to rank third in importance to the State's economy. If the State were to lose its oaks, it would be much less attractive to vacationers. Estimates of the commercial value of our oak forests as tourist attractions would be difficult to make and impossible to substantiate. It is of interest, however, to note that oaks of one species or another serve as primary food sources

Table 2. Forest Types on Commercial Forest Lands in West Virginia 1949*

Forest Types	Commercial Forest Area	
	Acres	Per Cent
Red oak	3,207,700	32
Chestnut oak	924,200	9
White oak	887,900	9
Oak types, total	5,019,800	50
Hard pine - oak	662,800	7
Oak-white pine	62,900	1
Oak a major component, total	725,700	8
All types including oak	5,745,500	58
All other types**	4,113,900	42
Grand total	9,859,400	

*Taken from Wray, 1952.

**Chiefly yellowpopular (2,070,700 acres) and northern hardwoods (1,526,000 acres).

for the following West Virginia game animals; white-tailed deer, wild turkey, ruffed grouse, raccoon, gray squirrel, and fox squirrel (Wilson *et al.*, 1951). They also furnish some food for black bear, snowshoe hare, red and gray foxes, and bobwhite quail. Of the game animals listed for West Virginia only opossum, cottontail rabbit, woodchuck, and skunk are reported to receive no nourishment from oaks. Any major reduction in our forests would be reflected also in a deterioration of fishing conditions in West Virginia's mountain streams. On the basis of nation-wide estimates each hunter spends an average of $79.49 annually and each fisherman $91.98 per year on these forms of recreation. In West Virginia, 400,000 people bought hunting or fishing licenses during 1957 (Mitchell, 1957). For the licenses alone they paid more than $1 million.

An industry committee[10] was appointed by the West Virginia Forest Fire Protective Association in 1954 to estimate the tangible annual income to the State from the harvest of oak products. This committee, under the leadership of John Tillinghast, forestry consultant, reported an annual income of nearly $20 million from this source. This evaluation was based on, "expected grade returns as determined from Forest Products Laboratory log grades, various public sources of log and lumber prices, and trade journals. These sources indicate that the average value of commercial oak lumber, air dried, is $90.00 per thousand board feet, f.o.b. sawmill. The value of mine timbers, pulpwood, veneer bolts, stave bolts, and other products is based on known prices paid by buyers for these products, delivered at the point of use."

The committee reported that their estimate included wages paid to 12,000 workers, stumpage paid to some of our 80,000 land owners, taxes paid to state, county, and local governments, as well as profits which go to the producers of oak timber. These items are all included because they would be lost to West Virginia if our oak stands were to disappear.

The so-called "southern hardwood forest," of which oak is a chief or valuable component, is not important to West Virginia alone. It predominates in several eastern, central, and southern states (Zon, 1924). In the delta region and bottomlands of the southeast, oak timber is very important commercially. The largest supplies of virgin and old-growth timber are principally in the deep south where Louisiana, Arkansas, and Mississippi contain more than one-quarter of the board-foot volume of standing oak timber in the country.

[10]The membership of the industrial committee consisted of: D. B. Bonebrake, Forester. Pocahontas Land Corporation; H. D. Bennett, Forester, Appalachian Hardwood Manufacturers, Inc.; R. C. Kemper, Forester, New River and Pocahontas Consolidated Coal Co.; C. L. Kinney, Forester, The Mower Lumber Co.; E. P. Shreve, Forester, New Ganley Coal Corp.; and J. F. Tillinghast, Consulting Forester, Tillinghast and Reed, Chairman.

The suitability of oak for a wide variety of uses including mine timber and railroad ties, veneer, flooring and tight cooperage, has helped to make it one of the most important hardwoods of the United States. Oak species have ranked fourth nationally among all woods cut for lumber (Betts, 1945).

All oaks are lumped together in estimating the values threatened by oak wilt because this disease attacks and kills oaks of all species. Some white oaks may die as rapidly as red oaks, although most of them die more slowly. White oaks that recover are not immune to later attack. Where our consideration has to do with eventualities, it seems justifiable to conclude that all oaks in North America are threatened by this disease.

It is now clear that oak wilt spreads and intensifies its losses more slowly than chestnut blight, Dutch elm disease, or white pine blister rust. All three of these diseases spread more rapidly when uncontrolled than oak wilt appears to be spreading now. Surveys have recently been made in Wisconsin counties where oak wilt seems to have been present for at least 20 years and where no effort has been made to check its spread (Anderson and Skilling, 1955). These surveys have shown that net volume has been reduced by 11 per cent through death of merchantable trees alone. The death of smaller trees within merchantable stands and in sapling stands has resulted in further losses in accumulated growth and removed considerable areas from oak production for an undetermined period. Individual forest areas ranging up to 60 acres have been rendered free of oak by the oak wilt. In discussing recent results of this survey (Anderson, 1958), Dr. R. L. Anderson recently commented, "If the rate of spread were to continue, all oak trees would be killed in from 70 to 100 years in our location." Given time and freedom to spread uncontrolled the oak wilt seems as capable of causing ultimately disastrous losses as have resulted from diseases that spread more rapidly. This difference in the time involved may enable us to hold the oak wilt close to its present comparatively low level of incidence in West Virginia.

The Symptoms of Oak Wilt

Both external and internal symptoms result from the presence of *Ceratocystis fagacearum* in oaks affected by oak wilt. Since other factors may occasionally induce similar symptoms (Dietz and Young, 1948), one cannot be entirely certain of the cause of the trouble unless *C. fagacearum* can be obtained in culture from the affected trees. Because this fungus is sometimes difficult to isolate from samples taken from diseased trees, especially during hot weather (Bretz and Morison, 1953), resampling and reculturing are sometimes required to determine whether

FIGURE 4. Characteristic symptom pattern in leaves of red oak trees affected by oak wilt. Note that the basal portion of affected leaves is the last to change color.

C. fagacearum caused the symptoms. For this reason, and to speed the treatment of disease suspects, the judgment of experienced men in the field is usually made the basis of diagnosis in routine control operations.

EXTERNAL SYMPTOMS

External symptoms of oak wilt in trees with mature foliage usually involve the water-soaking, bronzing or browning of the tips and margins of many affected leaves, with a sharp line usually separating the discolored portions from the normally green basal part of the leaf (Figure 4). Many, but usually not all of the affected leaves fall (Figures 5 and 6); some fall while almost entirely green, others after they become partly or completely bronzed or brown. One or more of the affected branches usually hold some green leaves longer than others do, so the crowns of oak wilt trees are seldom as uniformly brown as those of non-diseased trees that have been poisoned, girdled, or killed by lightning.

In the case of most members of the red-oak group, nearly the whole crown shows symptoms soon after the first symptoms appear, usually in the top of the tree. Members of the white-oak group frequently show foliage symptoms on only one or two branches at a time. The symptom branches may be dead the following year as the disease spreads rather slowly through the crowns of large trees. Small white oaks may show symptoms throughout the crown in the first year, as do red oaks.

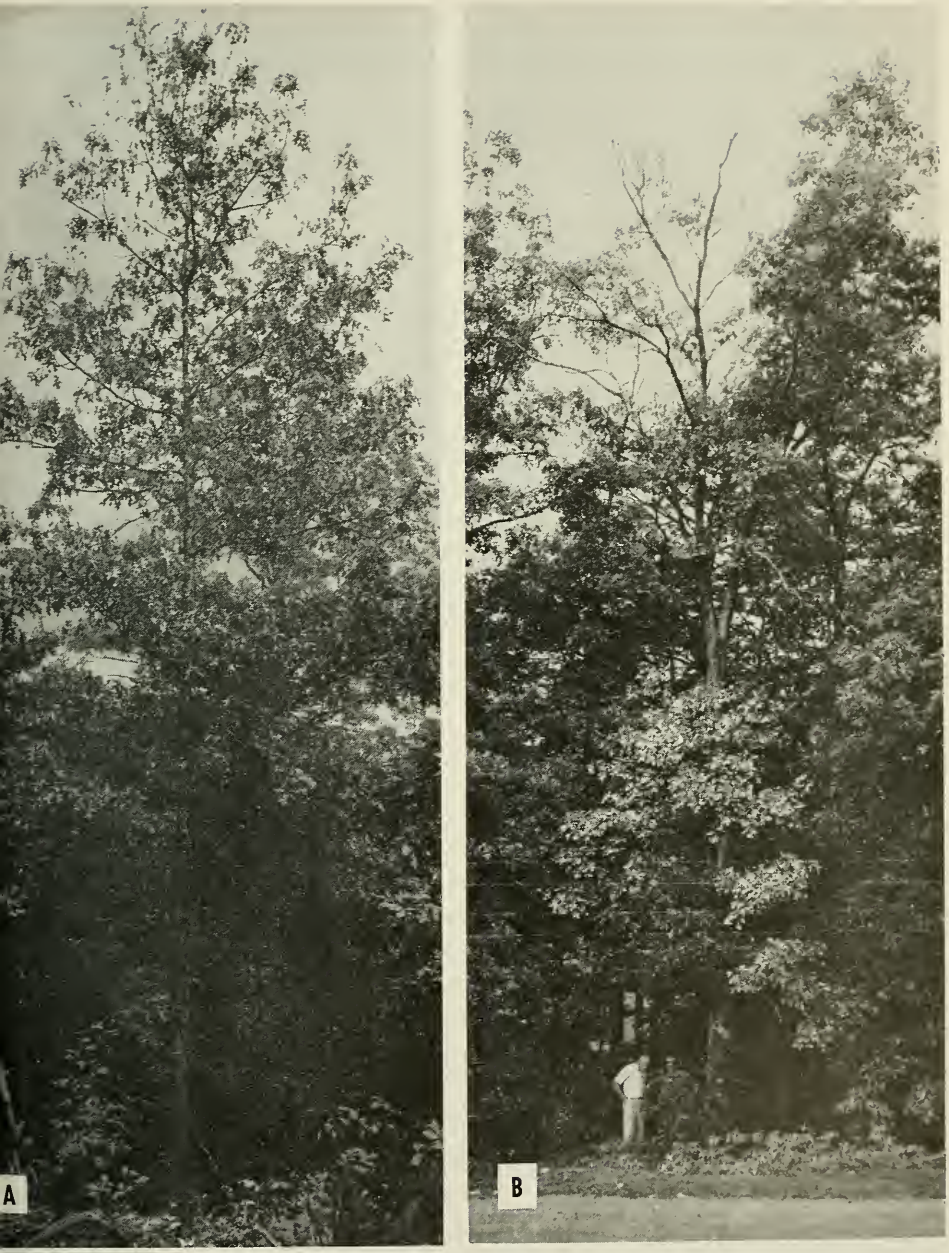


FIGURE 5. Symptoms of oak wilt shown in the crowns of diseased red oaks. (A) shows early stages of wilt and defoliation at the top of a young tree. (B) shows more complete defoliation of the top and also discoloration of the lower leaves.

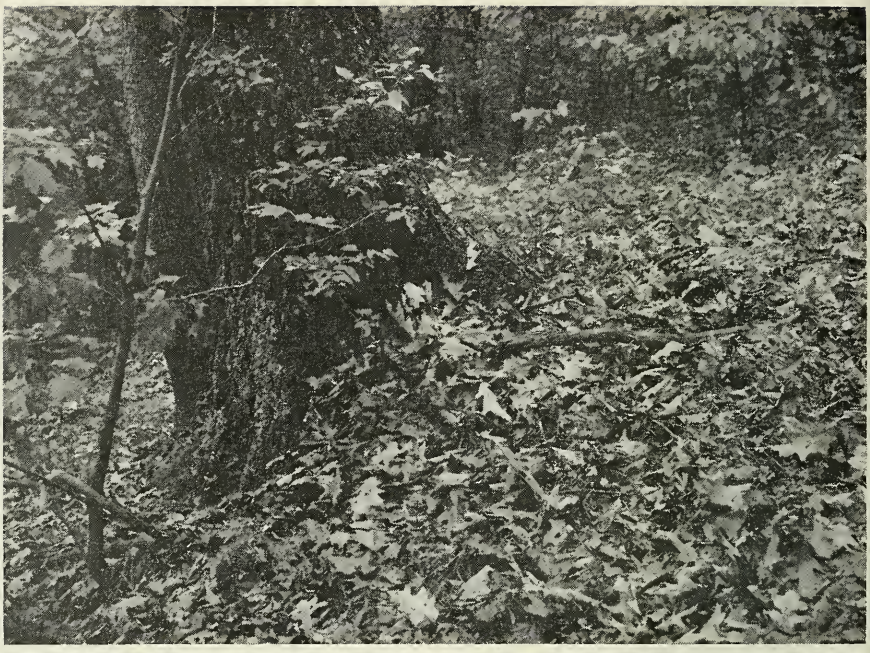


FIGURE 6. Accumulation of fallen leaves on the ground beneath an oak wilt tree. Some of these leaves fall while still completely green, many will be found entirely brown, but usually some will show the symptom pattern illustrated in Figure 4.

Diseased trees or branches usually hold some discolored leaves until autumn. This is a very helpful feature so far as scouting and control are concerned. During the first season all or nearly all of the leaves, twigs, and smaller branches of diseased trees of the red-oak group usually die, so that the whole tree may appear superficially to be dead after only a few weeks Some trees die during the winter. Usually, however, the trunk and larger branches of untreated diseased trees live through until the following spring or summer when they produce abundant short-lived sucker growth before they die (Figure 7). Death of the trunks of such trees coincides approximately with the death of the trunk suckers. Most oaks of the red-oak group die of oak wilt during the first or second year after symptoms appear, although all the leaves may die and many may fall within a few weeks after infection. The roots are the last part of the diseased tree to die, and the fungus may be isolated from infected roots after it can no longer be isolated from the rest of the tree. Most oaks of the white-oak group not only die more slowly, branch by branch in the case of larger trees, but they frequently recover. Such trees may serve as symptomless reservoirs of potential inoculum for a time after

FIGURE 7. Short-lived suckers may grow from the trunks and larger branches of defoliated trees either in the same year or in the year following defoliation.

apparent recovery (Jones, 1958). Trees that have recovered are subject to repeated infection and recurring disease.

The symptoms shown by the leaves on sucker shoots from diseased trunks, on sprouts from infected stumps, or by other succulent immature foliage differ from those just described for mature leaves. The apical and marginal portions of diseased succulent leaves blacken, curl and wrinkle, somewhat as though frost bitten, instead of turning bronze or brown. Such leaves remain attached to the stem. The early symptoms of succulent leaves may include pronounced wilting, whereas the more mature leaves usually appear rather to droop or to fold inward slightly.

Stambaugh and Nelson (1956) reported that trees infected late in the season may show no characteristic foliar symptoms of oak wilt, only a premature autumn coloration. Some of these trees die over winter without further symptom expression.

INTERNAL SYMPTOMS

Internal symptoms associated with oak wilt consist of an inconspicuous plugging of the large wood vessels affected and sometimes of a brown or a black discoloration affecting some or all of these vessels

and of the smaller cells adjacent to them. The water-carrying vessels may be prematurely filled by tyloses. These ingrowths from surrounding live parenchyma cells may expand far into the vessel cavity, decreasing its effective diameter and finally plugging it. Vessels filled with tyloses are easily seen to be closed when examined in cross section with a hand lens. Plugging by tyloses is almost always present in the vessels of stems affected by oak wilt, but similar effects may result from mechanical wounding and other causes.

Vascular discoloration is sometimes present in infected rings of stems affected by oak wilt. This is particularly true for diseased members of the white-oak group (Figure 8). Since there may be no discoloration of affected vessels in red and black oaks, this internal symptom is of less diagnostic value in the case of oak wilt than in some other vascular wilts such as the Dutch elm disease where discoloration is constantly associated with infection. Generally speaking, the internal symptoms of oak wilt are considerably less helpful in making a field diagnosis than the external symptoms.

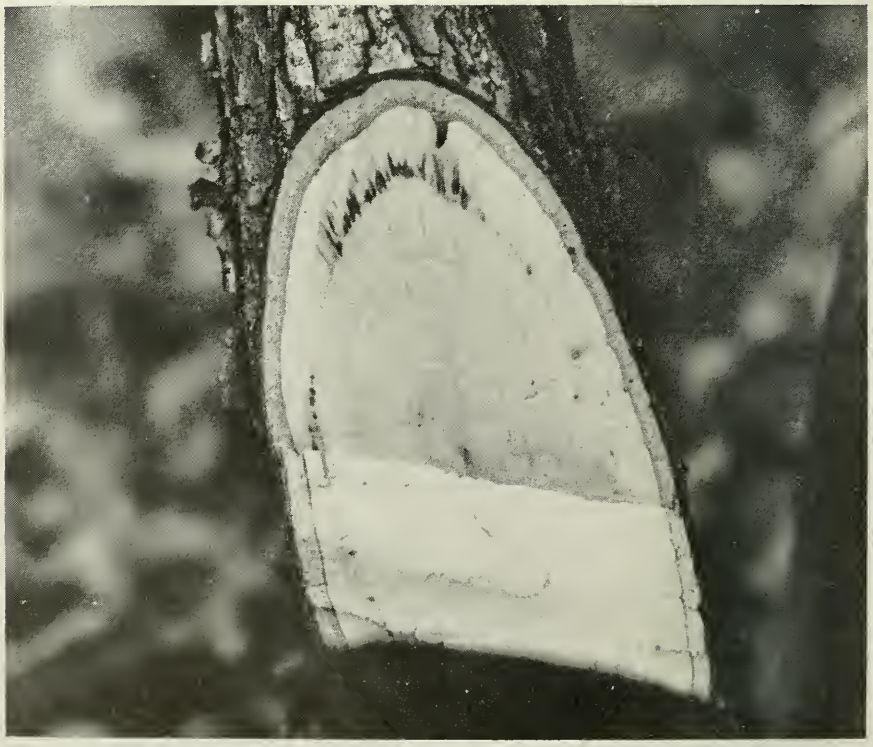


FIGURE 8. Discoloration of the infected annual rings may be an internal symptom of oak wilt in oaks of the white-oak group. It is seldom found in affected red oaks.

Indications and signs of disease in dead and dying trees may include bark cracks and pressure cushions (Figure 9) associated with oak wilt fungus mats, which often form beneath the bark (Figure 10) of trees recently killed by oak wilt (Leach, True, and Dorsey, 1952). Later, when the bark falls from the dead trunks of oak wilt trees, dark vertically elliptical patterns on the surface of the dead wood may mark the place previously occupied by oak wilt mats (Figure 11). Since similar dark patterns of the wood surface may result from other causes it is dangerous to interpret such "mat-scar" type patterns as diagnostic of the earlier presence of oak wilt in a dead tree.

The Oak Wilt Fungus, Ceratocystis Fagacearum (Bretz) Hunt

Information on the oak wilt fungus was reviewed earlier by Barnett and True (1955). The pathogen causing oak wilt (*Ceratocystis fagacearum* [Bretz] Hunt) was first named *Chalara quercina* by Henry (1944), on the basis of the conidial stage produced in culture. Sporulation in nature was unknown at this time. The brief summary of the principal characteristics of the fungus given below is adopted from Henry's description of the fungus as it appeared when grown on malt agar:

> Mycelium becoming gray to olive-green with occasional patches of tan; sterile hyphae sub-hyaline to brown, 2.5 — 6 μ in diameter; conidiophores not sharply differentiated from the mycelium, hyaline to brown, often tapering slightly toward the apex; conidia endogenous, hyaline, cylindrical, 1-celled, truncate at both ends, 2-4.5 x 4 — 22 μ.

Henry also stated that dark "sclerotia" are sometimes present in culture, being composed of loosly-knit cells. These structures were probably homologous to the fungus cushions now known to be produced in the cambial region of killed oak trees. No true sclerotia of this fungus are known.

The perithecia of *Chalara quercina* were discovered by Bretz (1952 c) in paired cultures. Because the fungus was known to be pathogenic to genera other than *Quercus*, Bretz discarded the species epithet and proposed for the oak wilt fungus the name *Endoconidiophora fagacearum*. The principal characteristics given in Bretz's technical description are as follows:

> Perithecia black, flask-shaped, with a spheroidal base 240-380 μ in diameter, almost completely embedded in the subiculum; walls membraneous to leathery; beaks 250-450 μ long, black, terminated by a fringe of hyaline hyphae; asci globose to subglobose, 7-10 μ evanescent, 8-spored; ascospores hyaline, 1-celled, elliptical and slightly curved, 2-3 x 5-10 μ, collecting at the ostiole in a sticky, creamy-white mass; heterothalic.

Bretz also included in his technical description the presence of "asexual, thick-walled, olivaceous to brown, polymorphic spores, 3.5 to

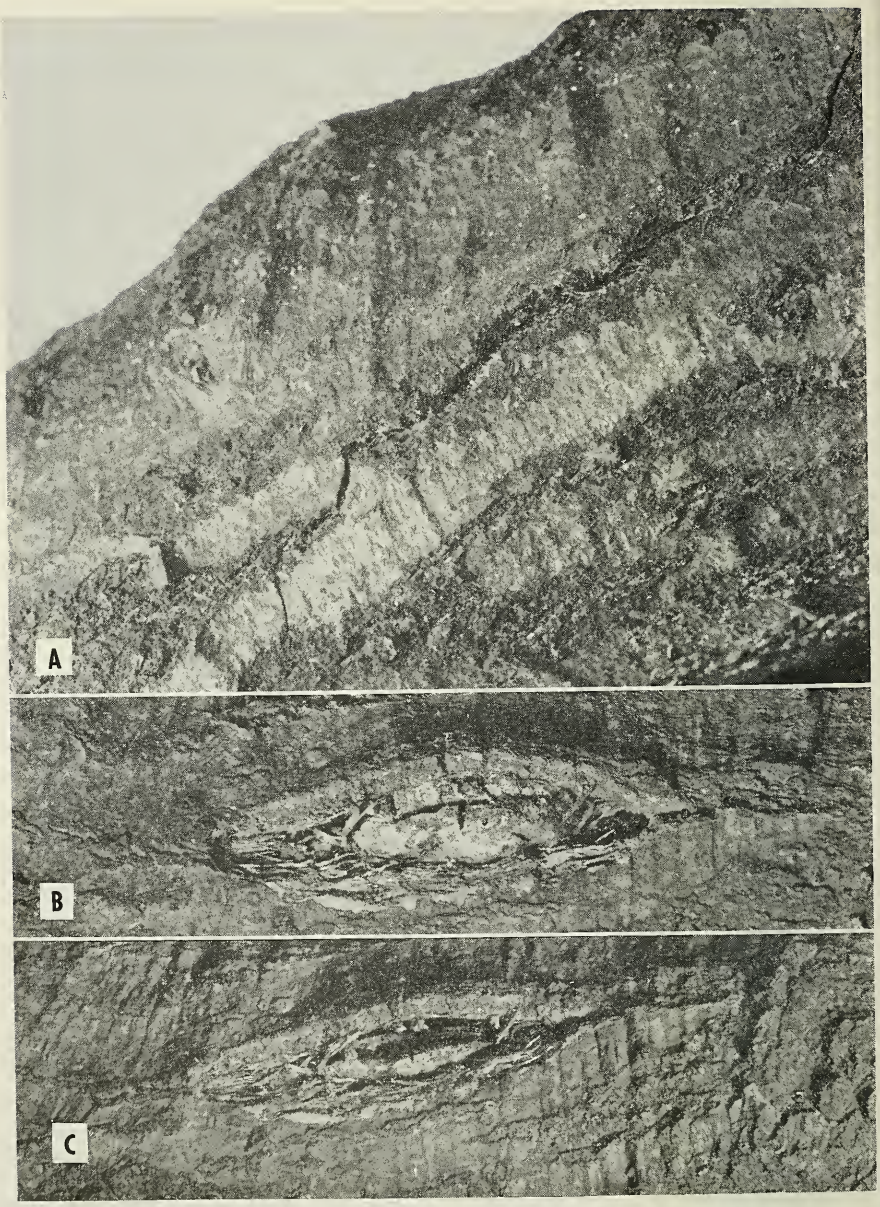


FIGURE 9. (A) Short cracks in the slightly bulging bark of affected trees are the first indication of the presence of fungus mats below. (B) The pressure cushions whose growth in the cambial region causes the bark to bulge and crack may be visible at the crack. (C) The pseudoparenchymatous tissue of the pressure cushions later shrinks as it dries out, leaving a wider opening.

FIGURE 10. Beneath the cracked or slightly bulging bark of dead or dying oak wilt trees may be found not only the central pressure cushions responsible for pushing up the bark but also the surrounding mycelial mats of the oak wilt fungus which develop between the bark and wood when a moist cavity is produced there by the growth of the pressure cushions.

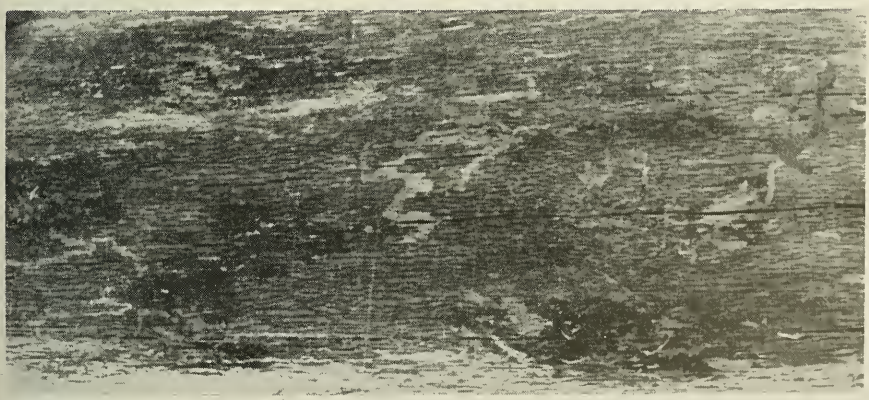


FIGURE 11. The dark remains of oak wilt mats and pressure cushions, "mat scars" on the trunks of trees killed by oak wilt. Since other fungi which inhabit dead trees may cause similar appearing structures, mat scars are not always reliable diagnostic signs of oak wilt.

5.5 μ wide and 5 to 20 μ long, formed endogenously and intercalarily—." He considered these as a spore form not previously described. These cells are either of rare occurrence or are not commonly recognized. It seems likely that these cells represent conidia or vegetative cells modified by age or cultural conditions, similar to those known for other fungi. It is doubtful whether they should be considered as a special form characteristic of this fungus.

Hunt (1956) has proposed a revision of the *Endoconidiophora-Ophiostoma* group of species under the revived generic name *Ceratocystis* Ellis and Halstead. Thus, the correct citation of the oak wilt fungus is now *Ceratocystis fagacearum* (Bretz) Hunt.

HETEROTHALLISM AND COMPATIBILITY

The perithecia of *C. fagacearum* were observed first by Bretz (1952 a) at the zone of merging mycelium between certain paired single-conidium cultures. Since perithecia did not form in other pairings or in non-paired single conidium and single-ascospore cultures, Bretz concluded correctly that the fungus is heterothallic. The nature of the heterothallic condition was explained by Hepting, Toole, and Boyce (1952 a, 1952 b). These workers selected for study 30 tissue cultures all originating from diseased oak trees in the Southern Appalachian Region. Only one of these tissue cultures produced perithecia without being paired with others. When eight single-conidium cultures were paired among themselves it was found that six belonged to one group and two to the opposite group. Applying the technique of spermatization, which involved the transfer of spores from one thallus to another, Hepting, Toole and Boyce (1952 a, 1952 b) established the fact that the conidia of each isolate may act as spermatia when transferred to a thallus of the opposite group. The latter thallus acts as a female and produces perithecia, usually within three or four days. Thus, it was demonstrated that each single-spore culture of *C. fagacearum* is hermaphroditic (bisexual) and that they fall into two groups, designated arbitrarily as compatibility type A and compatibility type B. These two types were found to occur in the Appalachian area in an approximate ratio of 1:1. The isolate found by Hepting, Toole, and Boyce (1951) to produce perithecia without being paired was later shown to be a mixture of A and B thalli and Boyce and Garren (1953) sketched the distribution pattern of each of the compatibility types within the affected tree.

It was then necessary to know whether both compatibility types were present in other oak wilt areas and to know whether geographic races of the fungus existed. To answer this question, 96 isolates were

obtained from eight states and were paired in spermatization tests[11] with standard cultures of known compatibility type. The cultures originated from Illinois, Iowa, Minnesota, Missouri, North Carolina, Ohio, Pennsylvania, and West Virginia. Briefly, the results showed that both compatibility types occurred in all eight states represented and there was no evidence of geographic races (Barnett, 1952 b).

During the 1952 season several isolates were obtained from each of 43 naturally-infected trees in West Virginia. All isolates from 23 trees were of the A compatibility type, while all isolates from 18 trees were type B. Both A and B type isolates were obtained from two trees (Barnett and Staley, 1953). From these results it was concluded that the two compatibility types occurred in West Virginia in a frequency of about 1:1, and that both types seldom occur in the same tree.

SPORULATION IN NATURE

Although the oak wilt fungus can be isolated from widely separated parts of the trunk, branches, and twigs at the time of initial wilting, workers have experienced difficulty in finding mycelium or spores in the vessels until after severe symptoms appear and tyloses have formed (Young, 1949; Fergus and Wharton, 1957; Struckmeyer, Kuntz and Riker, 1958.) It has been demonstrated that the endoconidia can pass through the vessels of red, white, and bur oak twigs that have not been plugged by tyloses (Young, 1949). Little or no mycelium has been found in the vessels at this time and it is concluded that the conidia are responsible for the rapid distribution of the fungus throughout the diseased tree (Young, 1949; Struckmeyer, Kuntz and Riker, 1958).

The production of new conidia within the vessels while they are still actively transporting sap apparently has not been observed, but it is certain that an increase in number of conidia occurs between the time of inoculation and the time when the fungus can be readily isolated from all parts of the tree. However, there is no reason to assume that the method of producing endoconidia in the sap (a liquid culture medium in itself) of the vessels is fundamentally different than their production within submerged liquid medium inside a test tube. In liquid media the spores readily form short conidiophores which immediately produce endoconidia.

It has been reported that during the period of severe foliar wilt the fungus penetrates the ray cells and begins to grow radially through them toward the cambium (Struckmeyer, Kuntz and Riker, 1958). Soon

[11]Spermatization involves the application of spores or mycelium of *C. fagacearum* to receptive mycelium of either compatibility group to attempt to induce the production of perithecia in the A and B test cultures (see also pages 47, 48, and 78).

after a tree dies of oak wilt the mycelium of the pathogen grows both radially inward toward the heartwood (Jones and Bretz, 1955; Englerth, Boyce and Roth, 1956) and outward across the cambium to penetrate the phloem region (True, Staley, Barnett, Leach and Dorsey, 1952). The conditions that influence its growth and longevity in wood and bark are not well understood, but it is believed that moisture, temperature, nutrient supply and competing organisms are important factors. If conditions are favorable, the mycelium may accumulate at local points in the cambium region. At these points the fungus begins to form its sporulating mats, (Curl, Stessel and Zuckerman, 1952; Kuntz, Parmeter, Ross and Riker, 1952; Hepting, Toole and Boyce, 1952 a) both on the outer face of the wood and on the inner face of the bark. Mats have also been found within the bark (Barnett, Staley and True, 1952; Fergus and Stambaugh, 1957).

The mats formed at the cambium arise as a layer of rather tightly woven mycelium extending over an area of several square inches around a central raised pressure cushion. Fresh mats are usually gray or tan with lighter margins (Figure 13). They are completely covered with conidiophores producing endoconidia in great numbers. The mats become darker with age and finally disintegrate. As many as 75 have been found on a single tree (Morris and Fergus, 1952). They occur frequently on trunks and larger branches and sometimes on branches as small as 1½ inches in diameter.

Near the center of each mat there usually develops a pair of thick, rounded or elongated, cushion-like structures, one of which is attached to the bark and the other to the wood (Figure 12). These cushions are formed back to back and, as they increase in thickness, they create enough pressure to raise and often to rupture the bark (Figure 13), thus creating a large lenticular, cave-like, moist chamber in which the fungus mats develop and the spores are produced.

The pressure cushions, the function of which was first recognized by Leach, True, and Dorsey (1952), have also been called "pads" by others (Fergus, 1953 a; Zuckerman and Curl, 1953), and more recently "pressure pads" (Fowler, 1958). These structures are composed mainly of large inflated, modified vegetative cells of the fungus. When the pressure surfaces of the cushions are separated they exhibit a net-like pattern of lighter reticulations that has been described as daedaloid. As the cushions dry out, the two members of the pair separate along the line of mutual contact, leaving the mat and cushion on the bark as the mirror image of those on the wood (Figure 12-A).

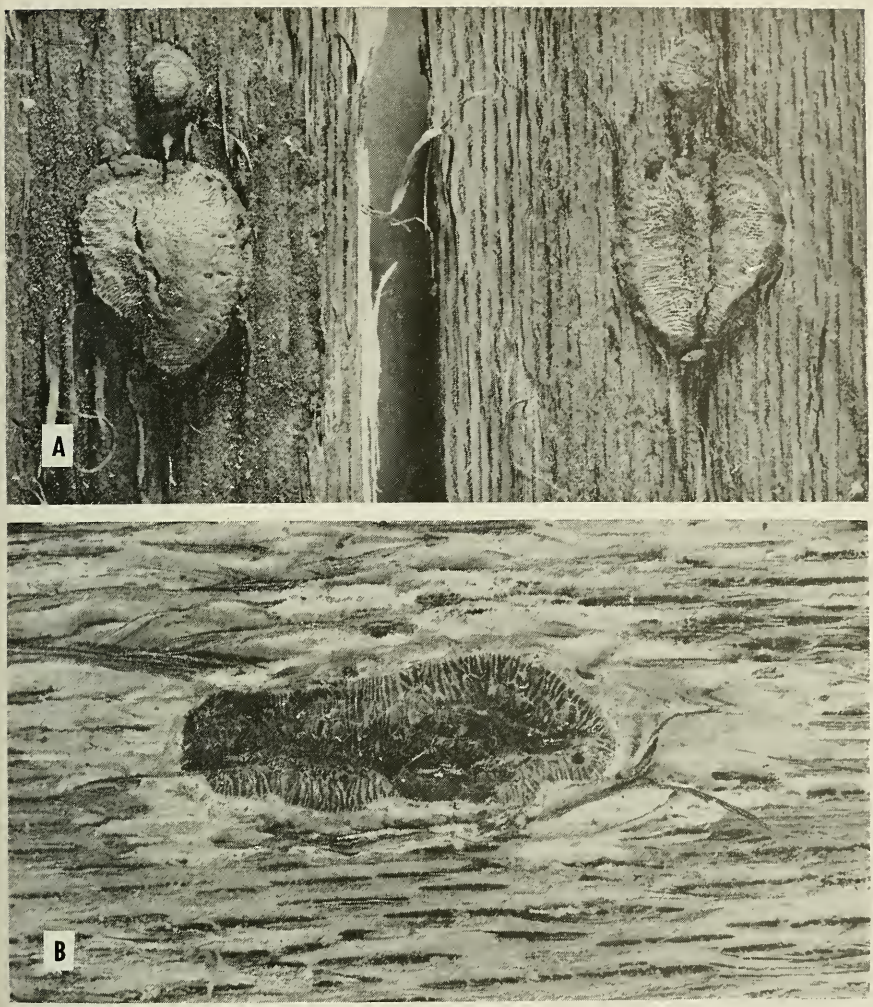


FIGURE 12. Sporulating mats of *C. fagacearum* showing the pressure cushions. A. The duplex nature of the cushion with one portion attached to the wood (left) and the other attached to the bark (right). B. Close-up of surface of one cushion where it was in contact with the other.

As soon as the fungus mats are exposed to the air by the cracking of the bark, they are ready for spermatization. This was determined by placing conidia of both compatibility types on different areas of the same mat (Barnett, Staley, and True, 1952). Fertile perithecia appeared in the area receiving conidia of the opposite type three days later. The cracks in the bark caused by the specialized pressure cushions open up a passageway through which a fruity odor, given off by the fungus mats,

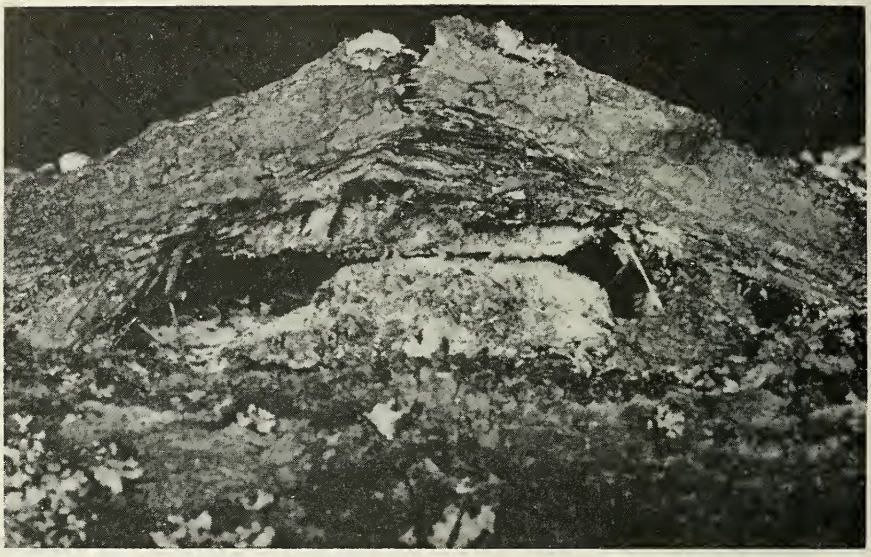


FIGURE 13. Side view of a pair of cushions seen through a bark crack. Note the break across the grain of the bark fibers resulting from the pressure exerted by the developing pressure cushion.

escapes to attract sap-feeding or fungus-feeding insects. These insects may carry conidia from one mat to another and act as agents of spermatization resulting in the production of many perithecia (Figure 14). This process is shown diagramatically in Figure 15.

ISOLATION AND IDENTIFICATION

Although the experienced observer can usually recognize oak wilt in the forest by the symptoms, positive confirmation can be made only by the isolation and identification of *C. fagacearum* from the wood. Isolations can ordinarily be made successfully from fresh samples from a diseased tree showing symptoms, provided the wood has not dried out too much and has not been invaded by secondary fungi. In extremely hot weather it is sometimes difficult to isolate the fungus from samples that would be expected to yield it (Bretz and Morison, 1953).

The methods of isolating the oak wilt fungus are similar to those used for isolating other wood-inhabiting fungi. Samples are usually cleaned and surface sterilized by a quick dip in 95 per cent alcohol and by flaming. Chips are then cut aseptically from the wood and placed on a medium suitable for growth of the fungus.

One of the more common media used for isolation was malt extract agar. This medium has the disadvantage that it does not favor early sporulation of the oak wilt fungus as it grows from the wood chips and

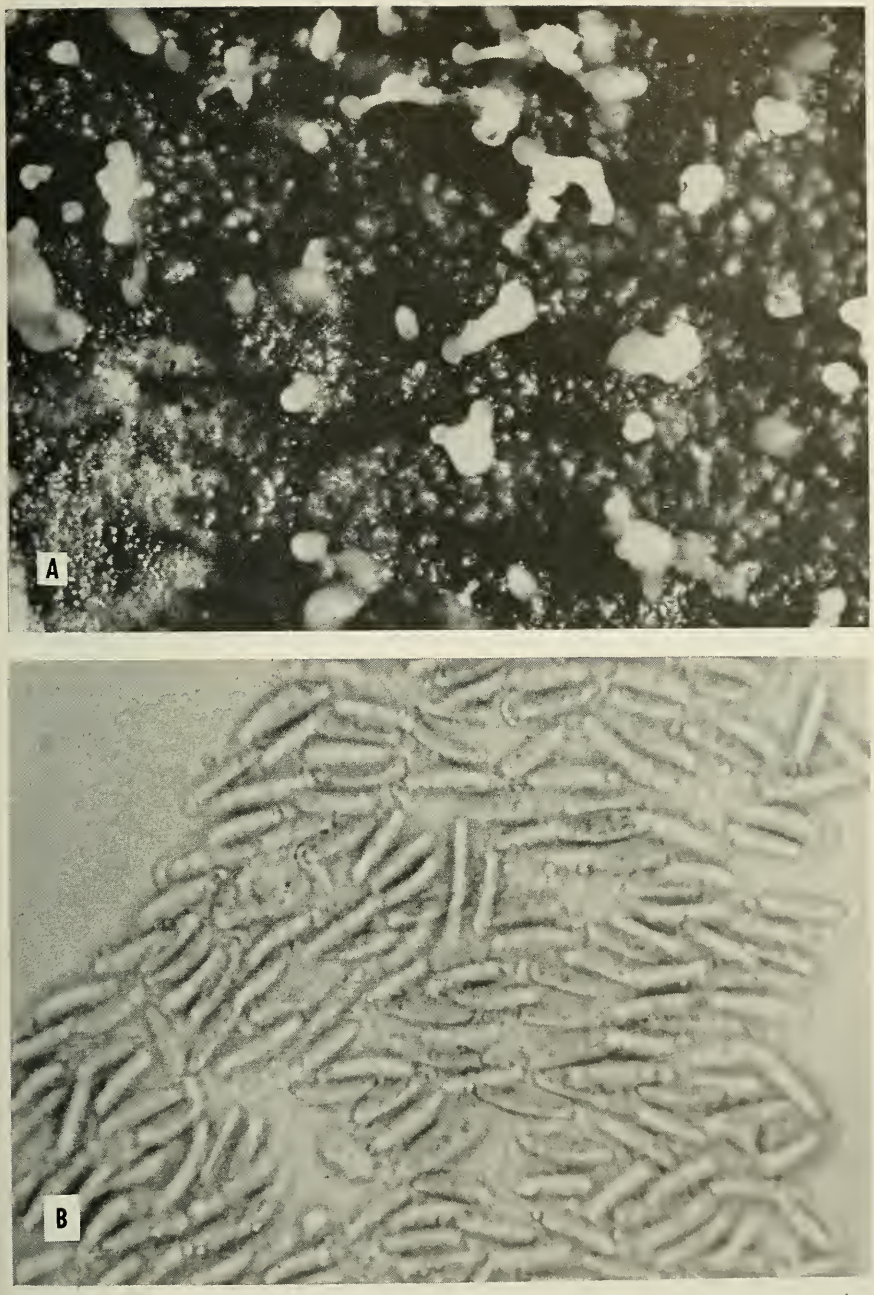


FIGURE 14. (A) A portion of a mature mat showing white masses of ascospores oozing from nearly-buried necks of dark perithecia. (B) Photomicrograph of a mass of ascospores.

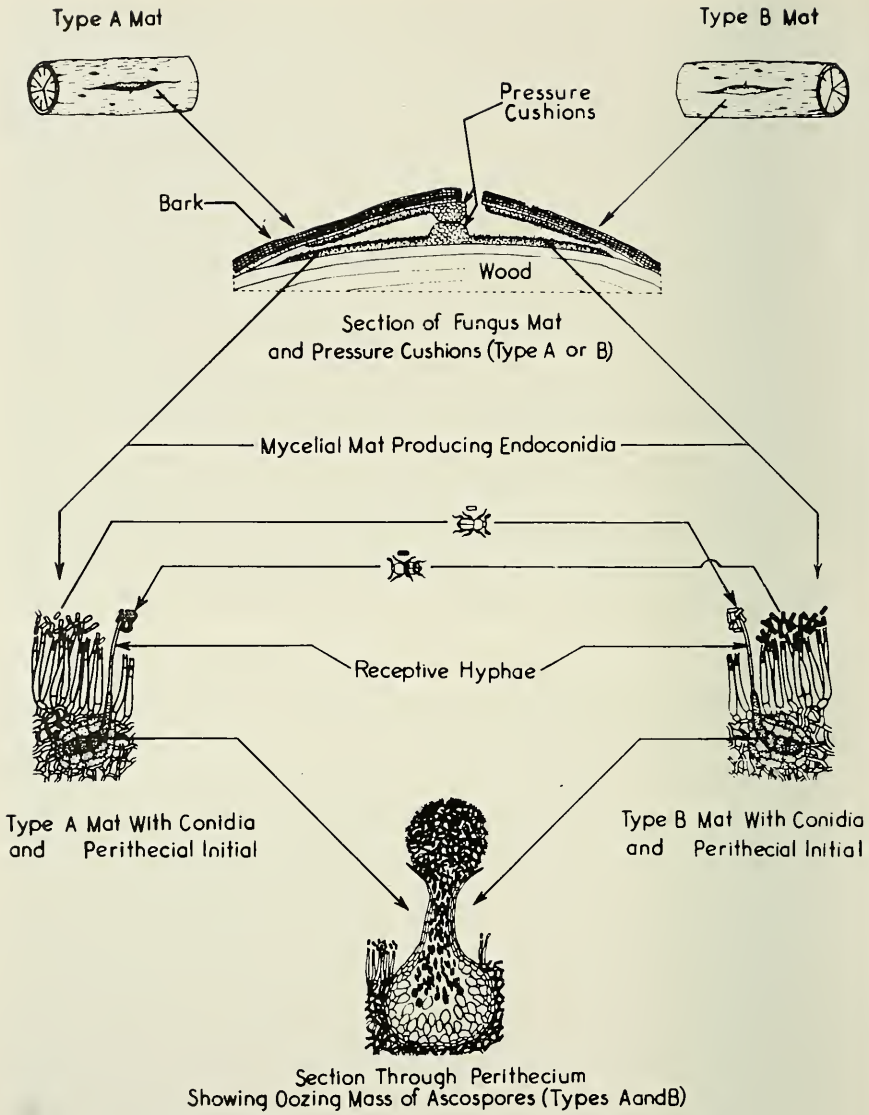


FIGURE 15. A diagrammatic representation of the role of insects in the spermatization of *C. fagacearum*.

it is seldom possible to make positive identification within eight to ten days. Even at this time it is often necessary to make water mounts of the mycelium to permit effective microscopic examination for endoconidia. This method is time consuming, and with the increased sur-

veys and inoculation studies, a quicker method of identification was highly desirable.

One new method suggested was to use a liquid medium in place of an agar medium (Barnett, 1952 a). The wood chips may be placed in test tubes of liquid medium and within four or five days mycelium from infected chips grows out into the liquid and sporulates quickly. Sterile distilled water can be used with equal satisfaction, but less mycelium and fewer spores are produced. This certain and rapid method of identification favors quick production of endoconidia. It was used as a standard isolation method by Bretz (1953) who pointed out certain advantages in its use for isolations in the field and for dry samples. He was able to recover the pathogen more frequently when using distilled water than when he used malt extract agar.

Although this liquid medium had certain advantages, it was necessary to remove the mycelium from the wood chip and examine it microscopically in order to identify the fungus. Extensive efforts to find a suitable agar medium that would permit quick easy identification of the fungus as it grew on the solid culture medium resulted in the recommendation of a glucose-phenylalanine agar having the following composition (Barnett, 1953 b):

D-glucose	3 g.
DL-phenylalanine	0.5 g.
KH_2PO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Micro element solution to give	
Fe and Zn, each	0.2 mg.
Mn	0.1 mg.
Distilled water	1 l.
agar	20 g.

The addition of vitamins, particularly thiamine and biotin, may increase the growth rate somewhat but the fungus will grow without this addition. The pH of the medium should be adjusted to about 5.5 to 6.0 before autoclaving. This medium has several advantages. The mycelium of *C. fagacearum* usually grows out from the wood chips within three to five days, frequently producing endoconidia immediately near the chip. The low sugar content limits the amount of mycelium and the phenylalanine, which is utilized slowly, decreases the growth rate of fast-growing contaminants. The mycelium can be examined directly with the 10 x objective of the compound microscope by placing the open Petri dish on the stage. After a little experience one can usually make positive identification in a few seconds. Using this medium, identifica-

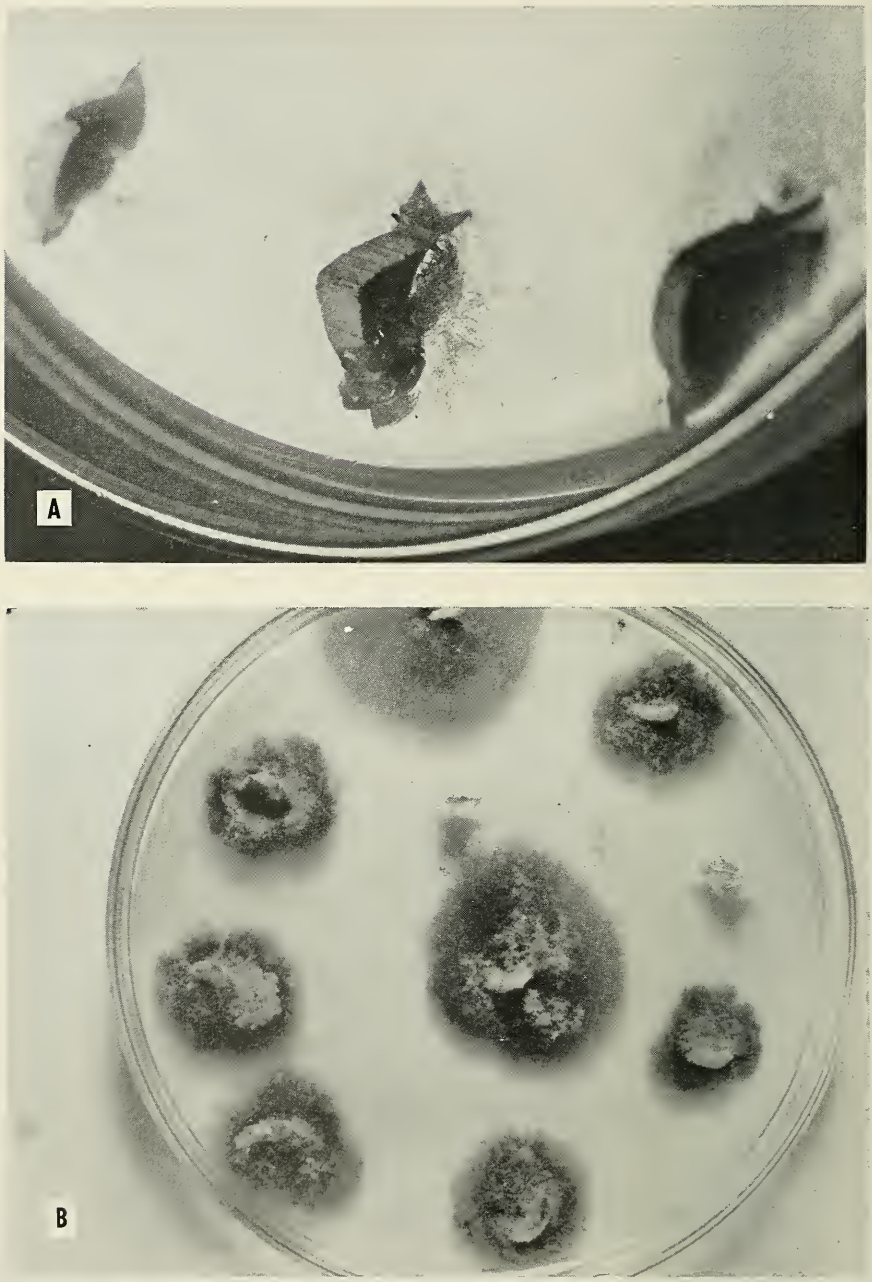


FIGURE 16. A. Sparse mycelium of *C. fagacearum* growing from an oak chip on glucose-phenylalanine agar. Age 5 days. B. Mycelium growing from oak chips. Age 9 days.

tion does not depend upon the presence of endoconidia, although they are helpful. The young hyaline mycelium growing from the wood chips (Figure 16) usually produces numerous curved or wavy branches which appear to be abortive conidiophores (Figure 17). These branches vary in abundance, but they are characteristic of *C. fagacearum*. This is the standard method for identification used in the laboratory at West Virginia University for several years and has proved reliable and satisfactory.

CYTOLOGY OF PERITHECIAL DEVELOPMENT

Culture studies had shown that fertile perithecia are formed regularly within three days after conidia of one compatibility type are

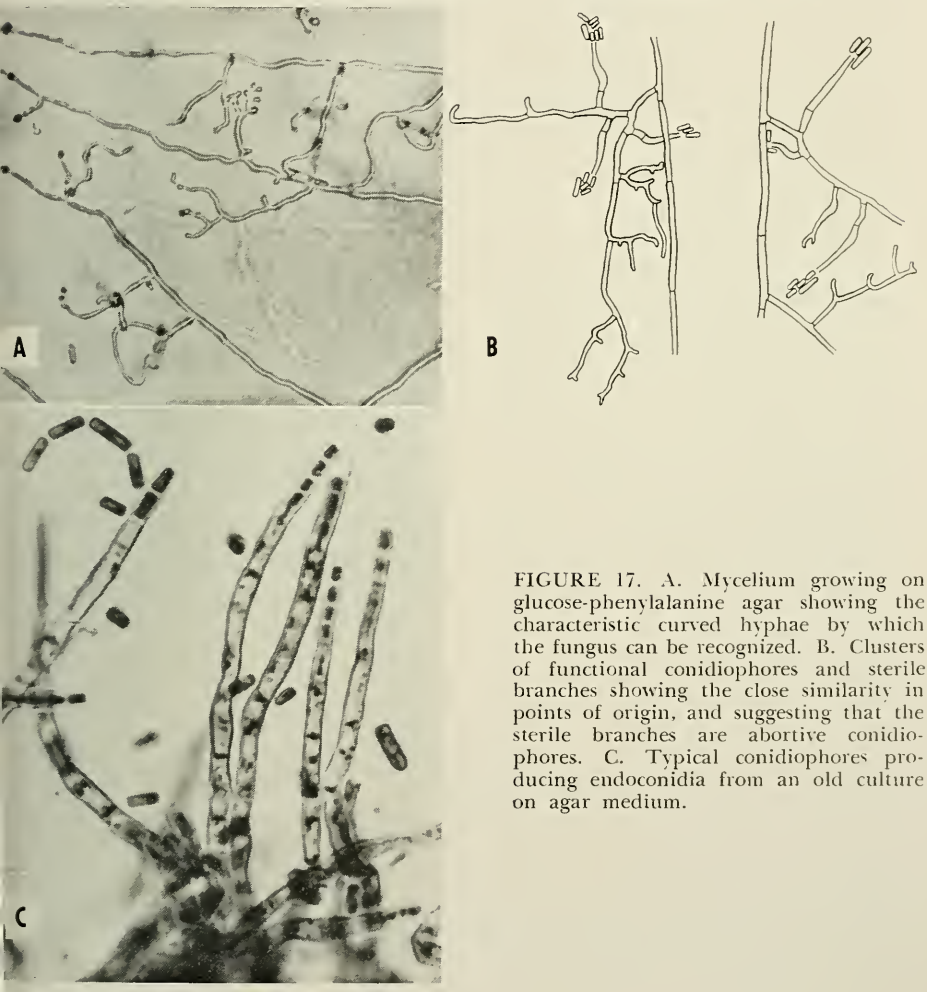


FIGURE 17. A. Mycelium growing on glucose-phenylalanine agar showing the characteristic curved hyphae by which the fungus can be recognized. B. Clusters of functional conidiophores and sterile branches showing the close similarity in points of origin, and suggesting that the sterile branches are abortive conidiophores. C. Typical conidiophores producing endoconidia from an old culture on agar medium.

transferred to a mat of the opposite compatibility type. In nature this transfer is accomplished by a vector according to the scheme outlined in Figure 15. The speed of development following spermatization suggested that perithecial primordia were already present in the mats. Bretz (1952 b) illustrated sections of perithecia, asci and ascospores, but no intensive cytological study of the development of perithecia was made until 1956 (Wilson, 1956 a, 1956 b; Tiffany, 1956).

The principal stages in the development of perithecia and ascospores, as described by Wilson (1956 b), are illustrated in Figure 18. The perithecial primordium begins its development in an unpaired culture as a thickened lateral branch (A), which becomes septate and narrowed toward the apex (G). The narrowed portion produces a few branches (E) and it is believed to function as a trichogyne, although the actual fusion between this structure and a conidium has not been observed. The basal enlarged portion becomes the ascogonium and is usually composed of three large, vacuolated cells (B,C,D). Branches arise at the base and by their growth surround the ascogonium by a loose sheath (B,E).

As early as four hours after spermatization cell division has begun within the ascogonium (C). These cells soon lose their identity and are replaced by smaller ones (F). About this time the base of the young perithecium begins to enlarge and chains of binucleate ascogenous cells become evident, extending inward from the wall (K). Karyogamy occurs first in the cells near the center of the perithecial cavity (K) and the formation of the ascospores (H,J) takes place rapidly. Neither the ascogenous cells nor the asci are walled. Formation of the perithecium neck begins about 20 hours after spermatization. The first ascospores are formed about 40 to 50 hours after spermatization and they accumulate in the central cavity from which they are forced upward through the neck canal. They collect at the ostiole in sticky masses. The ascospores are elongated and slightly curved (L), and each is surrounded by a thin layer of matrix.

VARIATION AND GENETICS

Variation among isolates of *Ceratocystis fagacearum* was first reported by Henry (1944) who noted that some cultures produced white mycelium after being held in storage for some time. Differences in the readiness with which cultures produced perithecia following spermatization were reported by Hepting, Toole, and Boyce (1952 a). These and various physiological differences among isolates have been observed many times by other workers. Some of these include differences in vitamin requirements (Figure 19) in sugar and nitrogen utilization, in re-

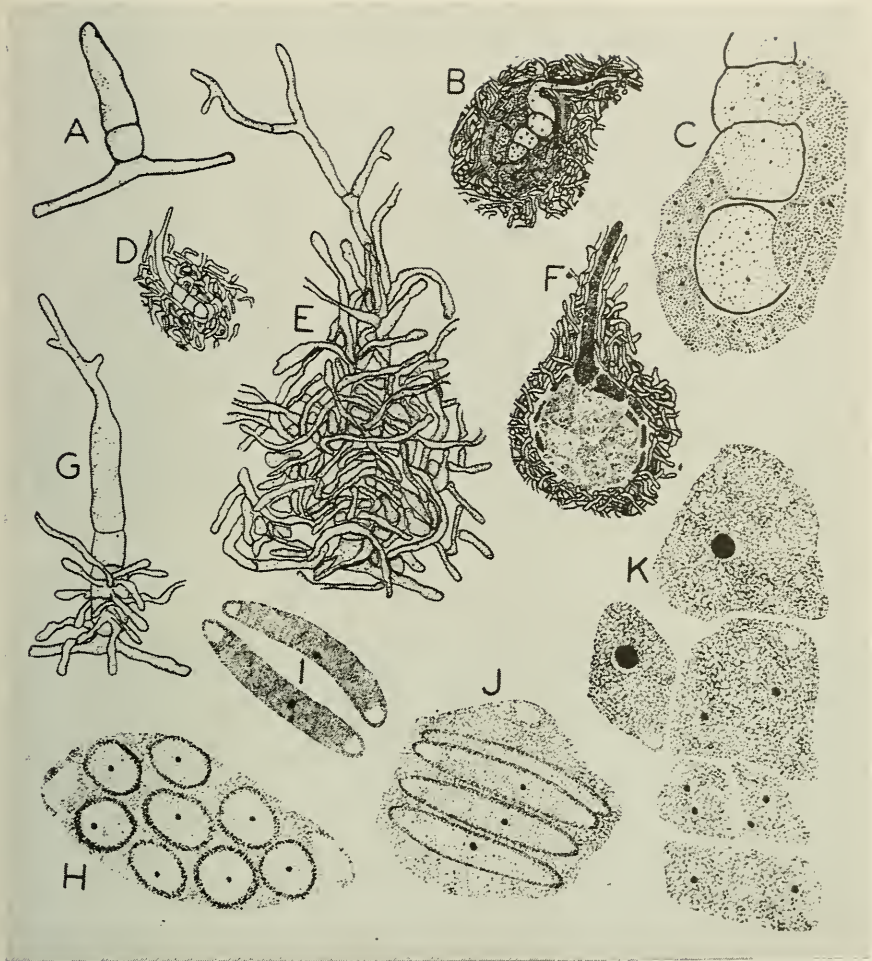


FIGURE 18. Stages in the development of ascospores of *C. fagacearum*. A. Upright, enlarged branch of the mycelium; B, perithecial primordium 4 hours after spermatization; C, three basal cells of ascogonium 4 hours after spermatization. Note the chain of cells being cut off by the cell next to the bottom; D, section of young ascogonium. E, ascogonium surrounded by sheath of mycelium. Note the branched trichogyne extending above the sheath; F, immature perithecium 8 hours after spermatization; G, developing ascogonium showing outgrowth of hyphae from basal cell; H, ascus showing end view of 8 developing ascospores; I, two uninucleate ascospores; J, ascus showing side view of ascospores; K, chain of ascogenous cells and two young asci with large nucleoli (diploid stage). (Wilson 1956).

sponse to temperature and in growth rate. It seems likely that isolates also vary in pathogenicity. This variability makes it difficult to compare physiological studies carried out by different investigators with one to a few different isolates.

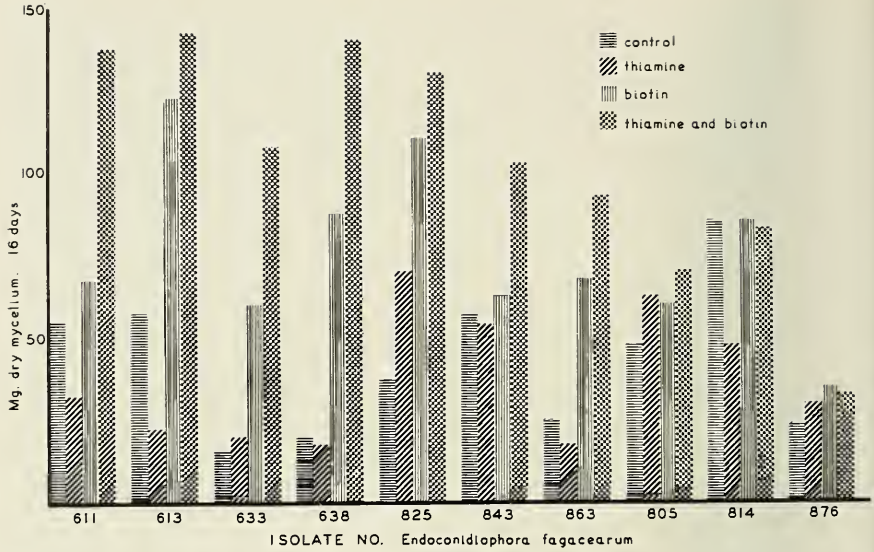


FIGURE 19. Variation in growth of ten isolates of *C. fagacearum* in response to the presence of thiamine and biotin in the medium. Isolates 805, 814 and 876 are light colored, unisexual mutants, and the others are normal, dark, bisexual isolates.

Little genetic work has been done on *C. fagacearum*. The first study of inheritance involved the use of a unisexual male culture that appeared in a transfer from a normal isolate. The culture (No. 805, A) was used in repeated reciprocal crossing attempts with a number of other isolates. It produced conidia capable of spermatizing normal cultures of the B compatibility type, but failed to produce perithecia in reciprocal crosses. It was concluded that this culture had lost the ability to act as a female and had become, therefore, a unisexual male (Barnett, 1953 a). The unisexual male was almost white, much lighter in appearance than the normally dark bisexual cultures. The light male A was crossed with a dark bisexual B isolate and single-ascospore cultures were obtained. The F_1 cultures were identified as to compatibility type and sexual behavior by back crosses. The F_1 cultures segregated as follows: 12 dark, bisexual B; 2 dark bisexual A; 6 light, male B; 3 light male A. The light mycelium and maleness segregated together and these factors may be linked. Dark mycelium segregated with the bisexual character (or femaleness). The factors for compatability segregated independently of sex or color of mycelium.

The loss of femaleness (the ability to produce perithecial primordia) appears to be common among cultures of *C. fagacearum,* but no

loss of maleness has been reported. Several mutations of dark bisexuals to light males have been induced by exposing conidia to ultraviolet irradiation.

In one dark bisexual culture from West Virginia (No. 645) white tufts of mycelium appeared which, when transferred, grew into a pure white, bisexual mycelium of the same compatibility type B as the parent. This was considered to be a true albino mutant (645 M). It produced both conidia and fertile perithecia in appropriate crosses. The perithecia were pure white, but were not numerous.

In 1953 isolates of a bisexual albino were obtained from an oak wilt tree that later produced white mycelial mats and pressure cushions (Barnett, True, and Brown, 1954). The mats and cushions were normal in every respect, except for color (Figure 20). This albino isolate (No. 2126) was also crossed with normal dark cultures and shown to be bisexual B in reciprocal crosses. When the albino A was crossed with a dark B, the F_1 progeny were either white or dark in a 1:1 ratio. Compatibility types again segregated independently of color of mycelium. Crosses between albino A and albino B yielded all F_1 albino cultures. A summary of the crosses and the results is presented in Table 3.

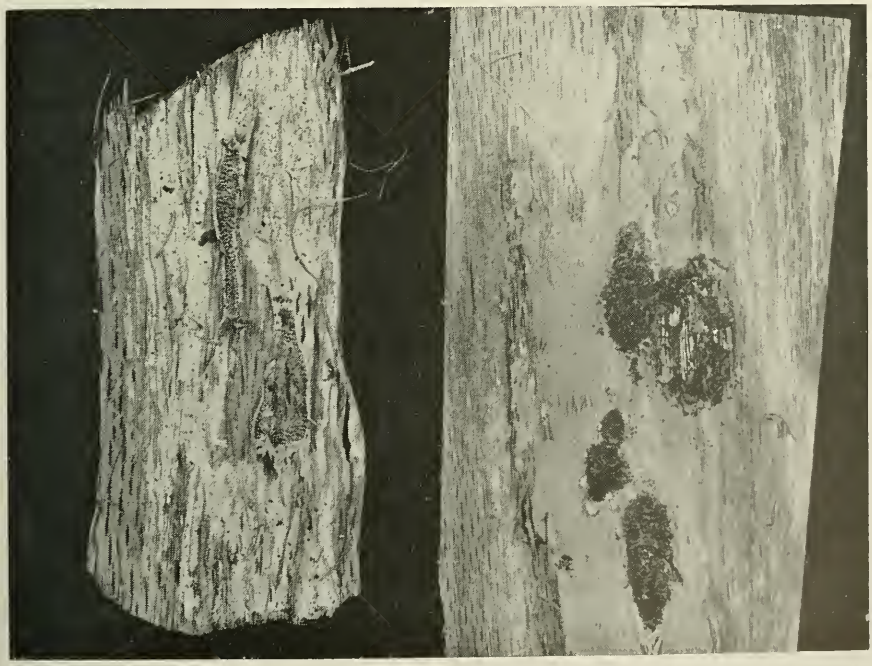


FIGURE 20. Sporulating mat of an albino mutant found on a naturally infected tree (left), compared to mat of normal dark fungus (right).

Table 3. Summary of Crosses of Isolates of *Ceratocystis fagacearum*

Male Parent A or B	Female Parent B or A	Perithecia	F_1 Isolates A and B
dark	dark	dark	dark
albino	dark	dark	dark and albino
dark	albino	white	dark and albino
albino	albino	white	albino
light male	dark bisexual	dark	light male and dark bisexual

The finding of an albino fungus in an oak wilt tree indicated that the fungus is variable in nature as well as in culture and that the albino was pathogenic. Inoculations of red and black oak trees showed that both albino mutants were pathogenic and that they were highly virulent. In fact, when a mixture of conidia from 645 M (albino mutant) and 645 (dark normal parent culture) was injected into trunks of oak trees, only the albino was recovered from 99 chips from 4 trees (see Table 4). The albino cultures have proved to be useful in inoculation and survival studies in nature.

Table 4. Recovery of Isolates of *Ceratocystis fagacearum* from Red or Black Oak Trees Inoculated with Mixed Isolates. F_1 Cultures Were Single-Ascospore Isolates from the Cross 645 Mutant Albino 2065. A and B = Compatibility Types

Isolates in Mixed Inoculations	Number of Trees	Number Positive Chips and Isolates Recovered
645 (dark B), 645 mutant (white B)	4	99 white B
645 mutant (white B), 2065 (dark A)	1	21 white B, 4 dark A, 11 mixed
	3	73 white B
F_1 1 (dark A), F_1 22 (white A)	1	20 white A
	1	38 dark A
	1	22 white B, 6 dark B, 4 mixed
F_1 3 (dark B),	1	14 dark B
F_1 24 (white B),	2	39 white B
	1	17 dark B
F_1 1 (dark A), F_1 3 (dark B),	2	59 dark A
F^1 22 (white A), F^1 24 (white B)	1	23 white B
	1	2 white A, 10 dark A
	1	1 white A, 10 white B, 2 dark A

COMPETITIVE DEVELOPMENT OF ISOLATES IN TREES

During routine sampling of oak wilt trees, even when multiple samples were taken, both compatibility types of *Ceratocystis fagacearum* were rarely isolated from the same tree. Hepting, Toole, and Boyce (1951) and Boyce and Garren (1953) reported one isolate that proved to be a mixture of A and B thalli. In West Virginia both A and B types were isolated from only two of 43 multiple-sampled naturally infected trees in 1953. Only type A was isolated from 25 trees, while 18 trees yielded only type B (Barnett and Staley, 1953). It was also reported that all isolates from the same mat were of the same type (Fergus, 1953 b).

The high percentage of oak wilt trees yielding isolates of only one compatibility type was unexpected and could not be explained by the use of naturally infected trees where the nature of the inoculum would in all probability consist of spores of both types. Attempts to obtain more information were made by using mixtures of conidia of both A and B types and ascospores in artificial inoculations of red and black oaks (Barnett and Staley, 1953). Later the experiment was extended, and identification of isolates made easier by use of albino cultures as components of the mixed inoculum (Barnett and Jewell, 1954). In these experiments, which covered two years, isolates were obtained from 25 trees artificially inoculated with 11 different mixtures of spores. From 6 to 40 isolates were obtained from each tree. Only 5 of the 26 trees yielded more than one type of isolate. A summary of the results of one of these experiments (in which 20 trees were used) is presented in Table 4.

These brief experiments showed that in the great majority of the oak wilt trees only one type of culture survived and was present throughout the trees, even though the inoculum consisted of a mixture of spores of different compatibility types or of dark and albino cultures. Thus, one may conclude that isolation of both compatibility types from a naturally infected tree means that the inoculum for that tree was mixed, or that the tree was inoculated twice with different types of inoculum. On the other hand, the isolation of but one compatibility type from a tree does not necessarily mean that the inoculum consisted of only one type. The reasons for the unexpected survival of only one type and its spread throughout most oak wilt trees are not known, but it seems likely to result from competition between thalli for survival within the tree.

LONGEVITY AND SURVIVAL

The question of longevity of the oak wilt fungus in logs, standing trees, roots, lumber, and even products became increasingly important when it became necessary to make specific recommendations for control. The first controlled experiments on longevity tested the effects of temperature and humidity on longevity of conidia (McLaughlin and True, 1952). Similar experiments later involved the effects of temperature and humidity on both conidia and ascospores (Merek and Fergus, 1954). Ascospores lived longer under most conditions than did conidia. Prewetting of ascospores reduced their longevity. Both kinds of spores lived longer under conditions of cool temperature and low relative humidity. For example, at 12° C. and 50 per cent relative humidity the conidia lived 139 days and the ascospores 232 days. At 31° C. and 75 per cent or higher relative humidity, both conidia and ascospores lived less than ten days. In general the results reported by Curl (1955) agreed with those of other workers.

An important discovery was made by Jewell (1954) who found that the conidia of *C. fagacearum* were viable after passing through the bodies of nitidulid beetles. Beetles were allowed to feed on fresh cultures of compatibility type A for a few days and then were removed and brushed with 95 per cent alcohol to remove surface spores. The fecal pellets were collected and placed in drops of sterile water on 7-day-old cultures of compatibility type B. Within three days, 67 per cent of these cultures produced fertile perithecia, showing that the conidia in the fecal material were alive and capable of spermatizing mats of the opposite type. The beetles having fed on spores of the oak wilt fungus could possibly serve as vectors under conditions unfavorable to survival of spores on the surface of their bodies. Using the spermatization technique, the receptive cultures actually acted as a "selective medium" screening out the contaminants which appeared abundantly when fecal pellets were placed on agar media.

Studies of the survival of the oak wilt fungus in felled logs of infected trees and in infected lumber have yielded variable results. This is not surprising since survival certainly is determined by temperature, moisture, competing organisms, and other factors. Spilker and Young (1955) successfully isolated the fungus from wood blocks after storage for 12 weeks at 5° C. and after 5 weeks at 25° C. Under conditions used by Englerth, Boyce, and Roth (1956) the fungus was isolated from air-seasoned and bulk-piled boards 22 and 24 weeks, respectively, after the logs were felled. They had been stored for six weeks before cutting. Isolations from sapwood having a moisture content below 20 per cent (dry wt. basis) were not successful.

The relation of fungus survival and insects was studied further by Stambaugh and Fergus (1956) who caged contaminated insects in polyethylene bags in the forest duff over winter. Conidia remained viable in or on dead or dormant nitidulid beetles for a period of 94 days and ascospores were viable after 151 days. It was concluded that insects coming in contact with ascospores on mycelial mats formed in the fall may be carrying viable spores at the end of their winter hibernation. These spores could serve as inoculum in early spring.

It is no great problem to keep cultures of *C. fagacearum* alive in the laboratory. Cultures on agar slants remain alive for many months. Some cultures in our laboratory have remained alive without transfer for five years, stored in screw-cap test tubes on malt extract-yeast extract agar at 5° C. Fergus and Cole (1955) showed that isolates of *C. fagacearum* stored under mineral oil for at least three years had undergone little or no alteration in regard to morphological, sexual or pathogenic characteristics.

PHYSIOLOGY

Temperature — *C. fagacearum* is favored by moderate or cool temperatures. It fails to grow and is soon killed at temperatures above 30° C. For this reason samples taken from oak wilt trees during hot weather must be kept cool and should be cultured as soon as possible. Bretz and Morison (1953) attributed the difficulty in isolating the fungus during the summer months of 1952 to unusually hot weather. They found that the fungus survived no longer than six days in twig samples held at 30° C.

In culture the fungus grows well at temperatures of 20° to 28° C., and makes fair growth at temperatures as low as 15° C. The extreme temperature range is roughly 5° to 32° and the optimum around 22° to 25° C. (based on reports of various authors). Growth of one isolate in a glucose-casein hydrolysate, liquid medium at temperatures of 10° to 30° C. is shown in Figure 21. Differences in reports by different authors can be attributed to variation in isolates, and to differences in media and environment.

Few conidia are produced above 28° C. and few perithecia are produced above 26° C. The optimum temperature for both is probably between 20° and 25° C. Stambaugh, Fergus, and Cole (1954) reported that a few perithecia formed at the extremes of 6° and 31° C. A maltose-casamino acids medium was more favorable for perithecium formation than malt extract medium.

Germination of conidia and ascospores occurs throughout the temperature range favorable to growth and is reported by Fergus (1954) to

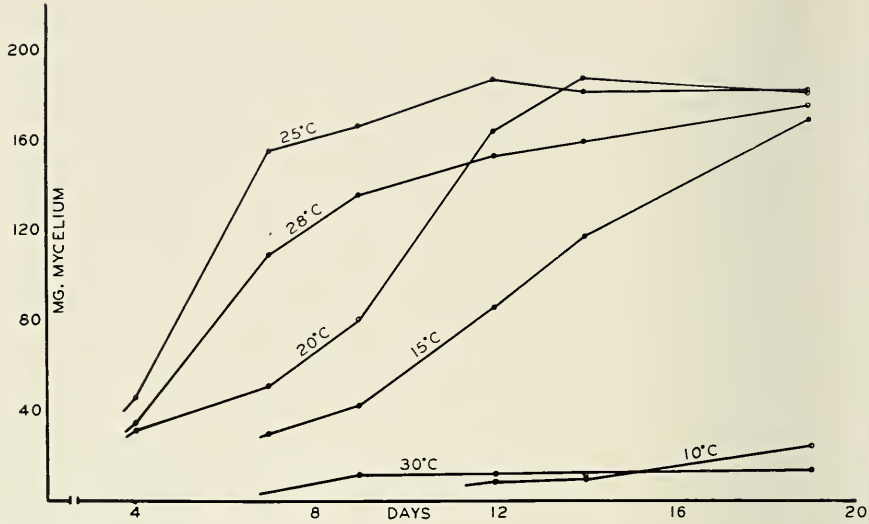


FIGURE 21. Growth of one isolate of *C. fagacearum* at different temperatures in liquid glucose-casein hydrolysate medium. Each culture flask contained 25 ml. of medium.

be from 3° to 33° C. Above 28 or 29° C. conidia and ascospores germinate by germ tubes that continue to branch and produce mycelium, while at lower temperatures conidiophores are formed immediately.

Vitamin Requirements — Early studies of the oak wilt fungus were made on media containing natural products, such as malt extract, potato-glucose, corn meal, and chestnut or acorn agars. Good growth was made on all of these media, but little was learned of the nutritional requirements of the fungus. Partial deficiencies for biotin and thiamine were reported by Barnett and Lilly (1952), who used a vitamin-free glucose-casein hydrolysate liquid medium to which the desired vitamins were added. Figure 22 shows the growth of one isolate studied soon after its isolation. This isolate had partial deficiencies for thiamine and biotin, but none for inositol and pyridoxine. Beckman, Kuntz, and Riker (1953) and Hoffman (1954) also studied vitamin deficiency and found some response to added inositol, but concluded that the deficiencies were negligible. Differences in results again suggest variability among isolates.

To determine the degree of variability among isolates in response to biotin and thiamine added to vitamin-free medium, 10 isolates were selected and grown at 25° C. (Barnett, 1956). Harvests were made after 10 and 16 days. The dry weights of the cultures after 16 days are shown

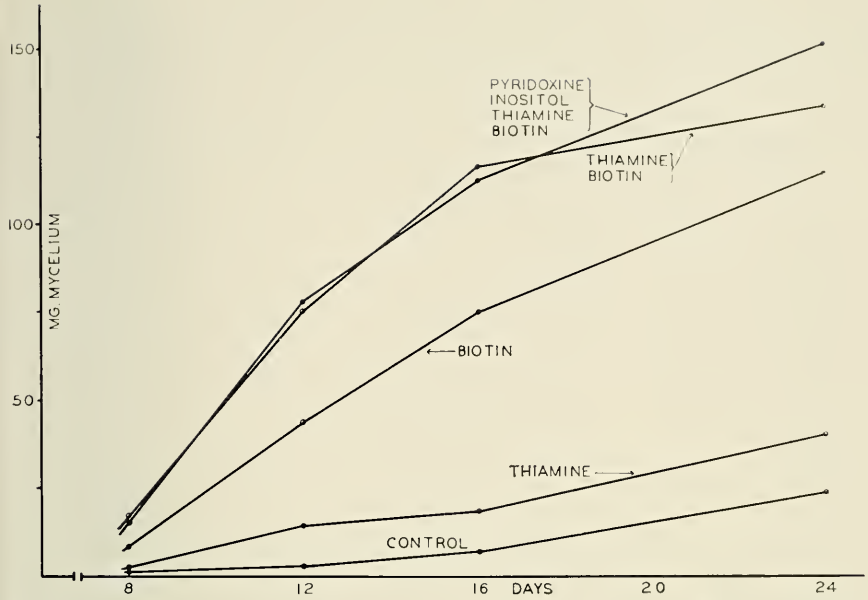


FIGURE 22. The vitamin requirements of one isolate soon after isolation grown in liquid glucose-casein hydrolysate medium. Note the partial deficiency for both thiamine and biotin.

in Figure 19. Much variation was found among isolates. Most of the isolates made better growth in media containing biotin alone or biotin and thiamine than in media with thiamine alone or no added vitamins. Isolates 633 and 638 showed the greatest deficiencies. Isolates 805, 814, and 876 showed little or no deficiency and made less total growth than other isolates tested. It is of interest that these three isolates previously had been shown to be unisexual males. This suggests that a pronounced change in physiology accompanies the loss of femaleness in the oak wilt fungus. The physiological change has not involved any reduction in pathogenicity.

Utilization of Carbon Sources — A number of carbon sources are readily utilized for growth in autoclaved liquid synthetic media. The utilization of 12 sugars by one isolate in a casein hydrolysate basal medium is shown in Table 5 (Lilly and Barnett, 1953). The most rapid growth of this isolate was made on mannose and sucrose, while glucose, cellobiose and maltose were also utilized readily. Little growth was made on sorbose and lactose. Other workers (Beckman, Kuntz, and Riker, 1953; Hoffman, 1954) have reported that other isolates readily utilize dextrin, xylose, and potato starch.

Table 5. Growth Expressed in mg. Dry Weight of *Ceratocystis fagacearum* in Liquid Media Containing each of 12 Sugars. Media Contained Casein Hydrolysate as the Nitrogen Source and were Sterilized by Autoclaving

Sugars	Days of Incubation			
	7	14	22	35
	mg	*mg*	*mg*	*mg*
D-glucose	45	164	—	—
D-fructose	23	53	157	137
D-mannose	82	171	83	—
D-galactose	30	78	126	—
L-sorbose	0	1	6	
L-arabinose	5	31	85	159
D-xylose	8	95	181	129
Maltose	25	156	—	—
Sucrose	63	141	—	—
Lactose	4	10	30	47
Cellobiose	37	147	—	—
Raffinose	23	56	88	80

The comparatively slow growth of *Ceratocystis fagacearum* on fructose, reported in Table 5, was unexpected, since the same isolate utilized sucrose well. In a search for the causes of these differences it was found that the method of sterilizing fructose greatly affected the growth of the oak wilt fungus. Temperature was also an important factor affecting growth on autoclaved fructose. The results of this experiment are presented in Table 6. Briefly, the inhibition shown by autoclaved fructose was not evident in sterile-filtered fructose. Inhibition by autoclaved glucose was evident only at high temperatures. There was no inhibition of growth by autoclaved sucrose (not shown in Table 6).

Utilization of Nitrogen Sources — The oak wilt fungus can utilize readily a number of nitrogen sources for growth in a liquid synthetic medium. Growth of one isolate in several media is presented

Table 6. Growth of *C. fagacearum*, Expressed in mg Dry Mycelium, in Liquid Casein Hydrolysate Media Containing Glucose or Fructose at 10 g per l. Sterilized by Autoclaving and by Sterile-filtration. Initial pH of all Media, Approximately 5.7 to 6.0

Temperature	Days	Glucose		Fructose	
		Auto-claved	Sterile-filtered	Auto-claved	Sterile-filtered
20° C.	15	190	200	74	261
25° C.	10	133	170	21	154
"	15	146	169	86	220
30° C.	15	11	30	3	55
"	26	26	57	3	56

Table 7. Growth of One Isolate of *C. fagacearum*, Expressed in mg Dry Weight, in Liquid Glucose Media Containing Various Nitrogen Sources at 2 g per l. The Initial pH was 6.0 Before Autoclaving

Nitrogen Source	Days of Incubation		
	7	10	17
No added nitrogen	trace	trace	trace
Potassium nitrate	trace	trace	trace
Ammonium sulfate	18	33	42
Ammonium tartrate	7	20	143
Casein hydrolysate	12	69	171
Asparagine	16	62	169
Aspartic acid	8	56	125
Glutamic acid	12	68	137
Arginine	19	79	171
Alanine	17	48	142
Glycine	7	55	100
Valine	20	49	76
Leucine	23	40	113
Methionine	10	36	56
Tryptophane	9	27	67
Cystine		19	57

in Table 7. There was little or no utilization of histidine, lysine, serine, threonine and tyrosine (not included in the table) by this isolate. The most rapid growth was made on casein hydrolysate, asparagine, aspartic acid, glutamic acid, and arginine. Nitrate nitrogen was not utilized. The results of other workers who studied the nutrition of this fungus were similar but varied in detail. (See Beckman, Kuntz, and Riker, 1953; Hoffman, 1954; White and Wolf, 1954).

It should be pointed out that malt extract agar has repeatedly been found inferior as a culture medium for *Ceratocystis fagacearum,* yet it has been used commonly. The reasons for its inferiority are not known, but the complex composition of malt extract may include a compound that inhibits the oak wilt fungus.

pH — No extensive careful studies have been made on the effects of pH of the culture medium on growth of *Ceratocystis fagacearum,* but most of the investigators have reported good growth within the pH range 4.0 to 7.0 (Figure 23).

Metabolic Products — The production of several metabolites by *Ceratocystis fagacearum* has been briefly reported by various workers. The production of pectic enzymes in culture was reported by Fergus and Wharton (1957) and these are believed to initiate the production of tyloses in the oak tree (Beckman, Kuntz, Riker, and Berbee, 1953). Young (1949) and Hoffman (1954) found a toxin produced in culture that caused wilting of tomato cuttings. Similarly, White and Wolf

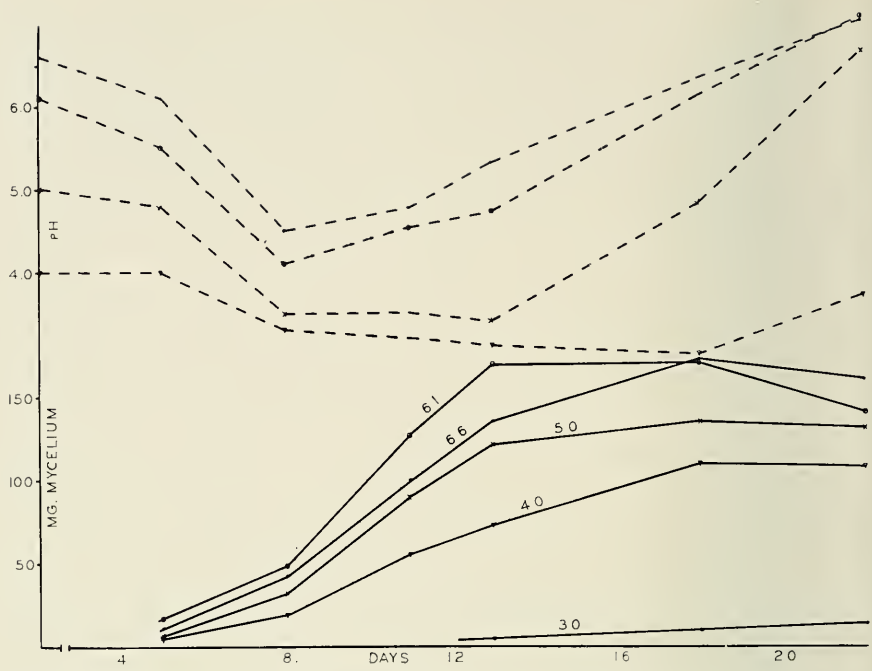


FIGURE 23. Growth and changes in acidity of liquid glucose-casein hydrolysate medium at different initial pH levels.

(1954) discovered that an alcohol-soluble compound produced in synthetic medium was toxic to leaves of *Quercus lyrata*. Another metabolic product of the oak wilt fungus in culture was believed to be a polysaccharide. The production of two polysaccharides was also reported by Wilson and Lilly (1958).

In culture, *Ceratocystis fagacearum* produces metabolic products having a fruity odor similar to that produced by fresh fungus mats in nature. It is possible to collect a volatile fraction boiling below 100° C. by distilling the culture medium in which the fungus has been growing for several weeks. Unpublished and incomplete studies suggest that acetaldehyde, ethanol, and ethyl acetate are present in the distillate. Saponification values indicate that about 60 to 70 per cent of the volatile distillate was an ester (calculated as ethyl acetate). Ethanol and ethyl acetate were found inferior to natural baits when tested as attrahents for various forest insects (Dorsey and Leach, 1956).

HOW THE PATHOGEN CAUSES WILTING

One of the earliest theories regarding the nature of the disease-inducing substance suggested that the oak wilt fungus produced, in the

vessels, a toxin which was transported upward to the leaves where it caused wilting. Early investigations showed that culture filtrates of *Ceratocystis fagacearum* did contain one or more substances which induced wilting of tomato plants and oak seedlings and leaves (Young, 1949; Hoffman, 1954; White, 1955). However, the presence of toxins or polysaccharides, also found in culture filtrates (White, 1955; Wilson and Lilly, 1958), did not explain the plugging of vessels by gums or tyloses.

Beckman, Kuntz, Riker, and Berbee (1953) suggested that the pathogen produced pectic enzymes within the vessels. These were thought to induce a weakening of the primary wall between the vessels and parenchyma cells, and to permit the formation of tyloses. These workers, however, did not demonstrate the presence of pectic enzymes either in the tree or in the culture filtrate.

Fergus and Wharton (1957) reported the production by *C. fagacearum* of ethylene and a pectic enzyme tentatively identified as pectin depolymerase in culture media containing potassium nitrate as the nitrogen source either with or without the addition of yeast extract. The production of this enzyme was greater on a medium containing pectin. The authors suggested that pectic enzymes produced by the oak wilt fungus in the oak tree act on the pectic lamellae so as to induce the production of "large molecular fragments" that collect in the vessels. These fragments, together with the tyloses formed, plug the vessels and induce wilting.

On the basis of the scanty information now available, it must be concluded that the nature of the wilting mechanism of *C. fagacearum* is largely unknown. While a pectic enzyme has been found in culture filtrates, none has been demonstrated in the diseased tree. It should also be pointed out that not all of the symptoms of oak wilt can be induced in a healthy tree by merely cutting off its water supply. It seems likely, therefore, that some factor other than a simple mechanical plugging of the water-conducting vessels alone plays some part in bringing about disease symptoms.

The Transmission of Oak Wilt

Oak wilt transmission has been termed *local spread* when newly infected trees are found within 50 feet of previously diseased oaks which are presumed to be the source. Some local spread is believed to occur underground through pre-existing root grafts which often unite the root systems of closely related oaks standing nearby. This type of local spread is termed *root-graft spread* in contrast to *overland spread*. The transmission of oak wilt for distances greater than 50 feet is called *long-*

distance spread and is considered to occur always overland. Although local spread of oak wilt occurs sometimes through root grafts, it seems likely that overland spread may result in local as well as long-distance transmission of oak wilt.

LOCAL SPREAD

The first contribution to our knowledge of how oak wilt is transmitted was the information that *Ceratocystis fagacearum* may spread from the root systems of diseased trees into those of adjacent healthy trees through pre-existing root grafts (Kuntz and Riker, 1950 a; Beckman and Kuntz, 1951; Riker, 1951). This root-graft spread contributes to the local intensification of the disease near infection centers initiated as the result of long-distance overland spread. For some time it was considered that all or nearly all local spread occurred through these root grafts which were found so prevalent in some areas in Wisconsin.

The existence of root grafts (Figure 24) through which local spread could take place was also demonstrated in the Appalachian region (True, Judy, and Ross, 1955), but here local spread was found less frequently (Staley and True, 1952; Boyce, 1957). In West Virginia 73 oaks, including members of the red-oak and white-oak groups growing on a wide variety of sites in the northern counties, were killed with sodium arsenite, formulated as recommended by Wisconsin workers (Pessin, 1942). This was applied with a poisoning axe in holes 1.5 to 2 inches apart around the base of the tree. Trees received 6 to 7 ml of the solution per diameter-inch. At only 8 of the 73 poison sites did adjacent healthy trees show effects from the sodium arsenite applied to the center tree of the plot. From 1 to 4 satellite trees were killed or injured at sites where poison spread to affect nearby trees. In the Appalachians a proportionately larger number of initial infections resulting from long-distance spread was found, and overland spread came to receive primary attention in this region.

It is now recognized that local spread need not always be the result of root-graft transmission. Agencies responsible for overland spread could conceivably carry the disease to healthy trees close by. Early investigators had suggested that root-graft spread might occur for distances up to 50 feet, although in most cases primary root-graft connections were shown between trees standing less than 25 feet apart (Kuntz and Riker, 1950 a). Studies in the Appalachian region revealed few, if any, known cases of primary root-graft connections between trees more than 30 feet apart. By far the greater number of nearby trees injured stood within 10 feet of the treated tree. The most distant of the trees so injured in West Virginia studies was 26 feet from the treated tree (Figure 25).

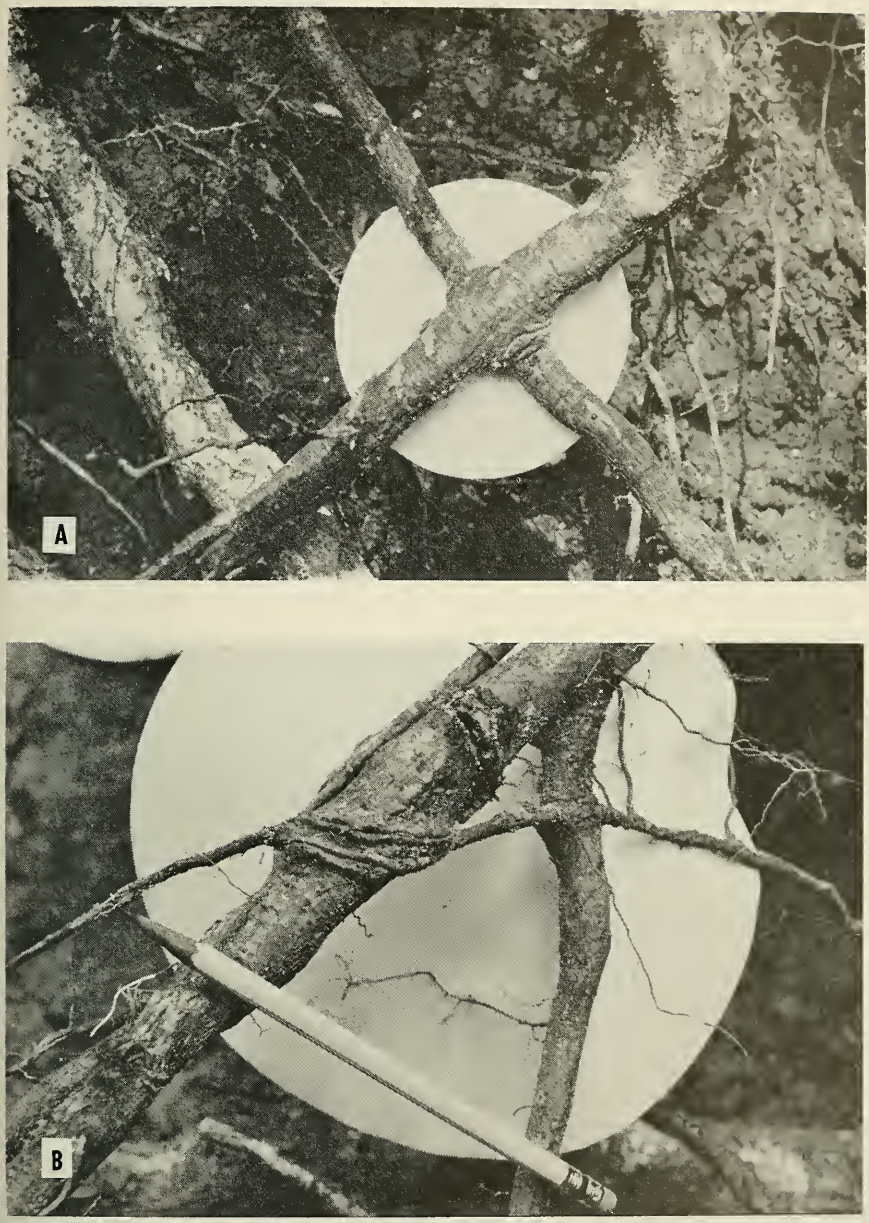


FIGURE 24. The roots of healthy oaks often graft together if they grow in close association. A. Where these root grafts involve the union of the wood vessels of the root of one tree with those of another, it is sometimes possible for dyes, poisons and for fungi to pass from the roots of one tree into the roots of the other. The spread of oak wilt in this manner is called root-graft transmission. B. Such grafts seem not always to be limited to larger roots but may involve roots of different ages and sizes.

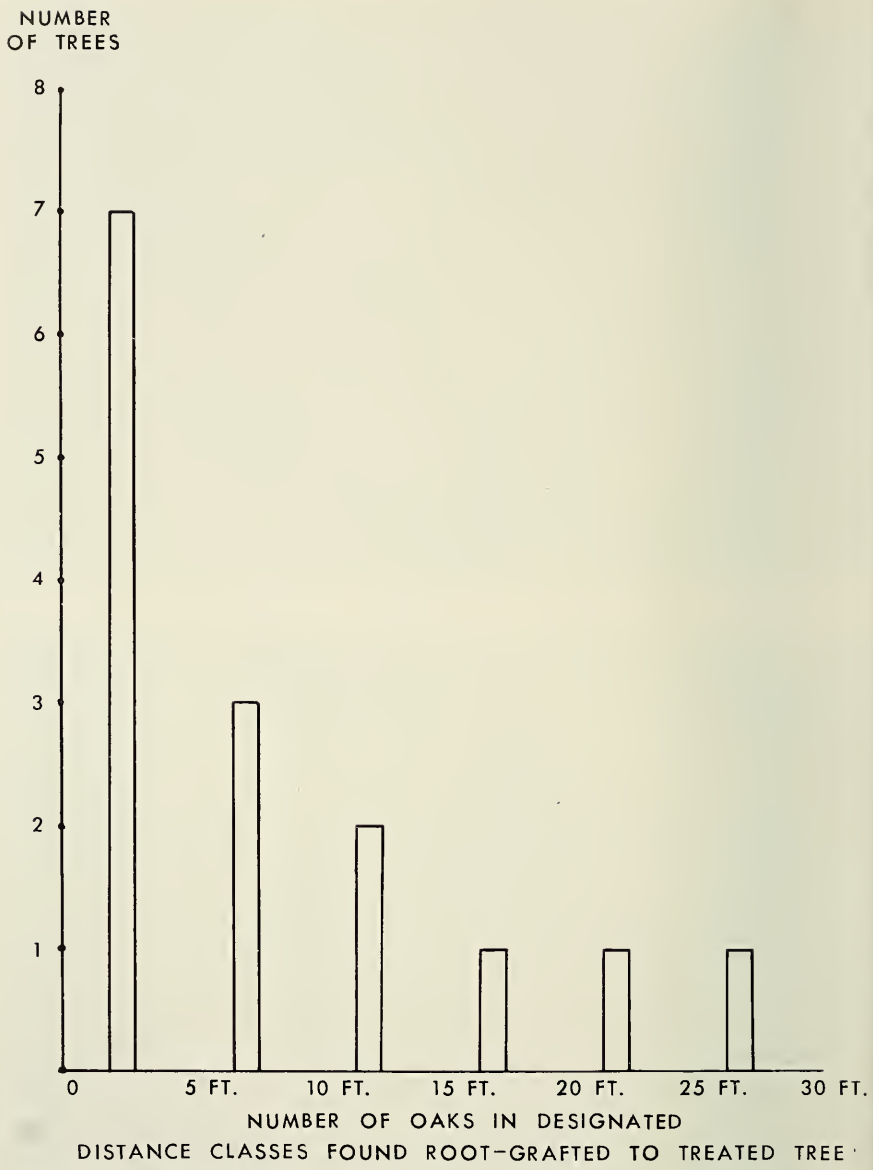


FIGURE 25. Distances between oaks shown to be united by root grafts in West Virginia studies. Dyes and poisons injected into one tree or stump were sometimes later found in the above-ground parts of one or more trees nearby.

Studies made in the deep sandy soils near Wisconsin Rapids showed that in the dense and comparatively pure stands existing there, the roots of closely related oaks made up a linked network uniting many

individual root systems (Beckman and Kuntz, 1951). Given time for secondary spread, the disease could be disseminated in this fashion for indefinite distances. Experimental work at Wisconsin Rapids revealed one case in which 37 individual trees shared a united root system. Under such conditions it did not seem surprising occasionally to find as many as 20 diseased trees appearing close around a single isolated oak wilt tree first diseased the previous year. Fortunately nothing comparable to this has been found in West Virginia or in other Appalachian states. Studies made in West Virginia with dyes and poisons have suggested that from 10 to 20 per cent of our oaks of the red-oak group are root-grafted to nearby susceptible trees. It is acknowledged that the methods used cannot be expected to reveal all root-graft connections uniting trees within experimental plots.

Current control practices in West Virginia include no measures expected to prevent root-graft spread to nearby trees. Under these conditions local spread (to trees within 50 feet) has occurred at least once in 11 per cent of 572 three-year-old infection centers, in 10 per cent of 880 two-year-old infection centers, and in 7 per cent of 681 infection centers during the first year after the diseased trees were found (Gillespie and Craig, 1959). Experience in West Virginia indicates that few infection centers show initial local spread after the fourth or fifth year (Gillespie and Craig, 1959) and that further local spread is unlikely at any infection centers where no local spread has taken place in this length of time. The conclusion is that local spread occurs in West Virginia, but that its importance (and that of root-graft spread which contributes to it) is much less here than early work in the middlewest had suggested.

Boyce (1957) presented data on the distance of 224 new oak wilt trees from the nearest dead standing oak or recently cut oak stump. Assuming that the dead, standing trees or cut stumps had died of oak wilt, he recorded the number of newly diseased trees found in each of six 10-foot distance classes from the assumed source. He lumped together the trees found from 60 to 100 ft. to give a total of seven classes. These data are weak numerically, but their probable validity is based on the reasonable assumption that in these areas the dead trees and cut stumps represented trees which had previously died of oak wilt. The data obtained are the best available at present and they seem suitable as a point of departure for discussion. When these figures are plotted in their original distance classes, curve (a) in Figure 26 is obtained. This curve indicates that fewer new trees were found in the 1- to 10-ft class than in the 11- to 20-ft. class, but for the succeeding distance classes, the trend was generally downward. This initial rise to the 11- to 20-ft.

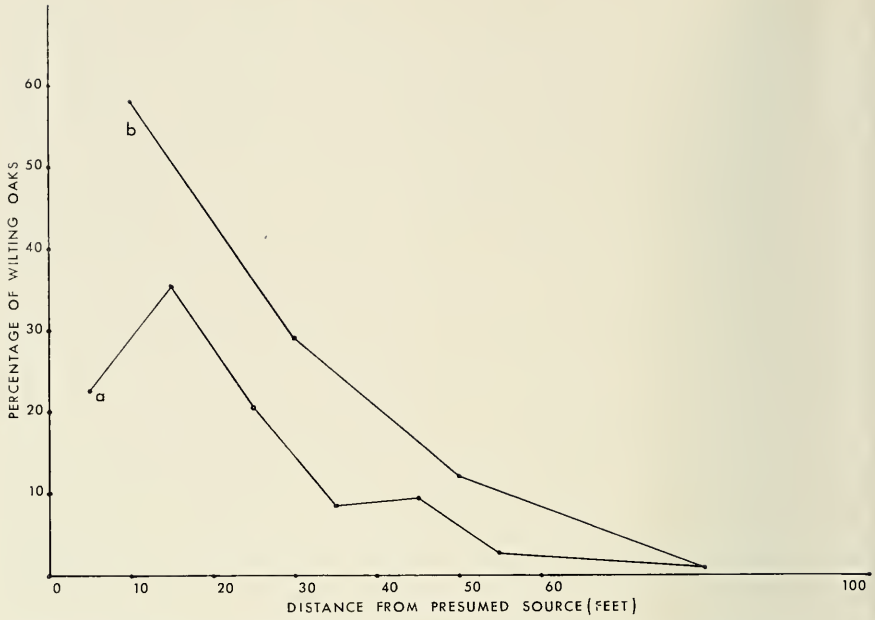


FIGURE 26. The percentage of total wilting oaks found within designated distance classes from presumed sources of local spread in North Carolina (Boyce, 1957).

class, not explainable on the basis of Figure 25, which is based upon West Virginia poisoning data, could be accounted for by the fact that a single large oak often dominates and completely suppresses smaller trees growing closer than 10 feet from it. Boyce could well have been dealing with larger trees than those utilized in West Virginia studies, where poisoned trees ranged in diameter from 5 to 13 inches.

When Boyce's data are regrouped into 20-ft. distance classes, curve b, Figure 26, is obtained. This curve drops off at a rather uniform rate, but it does not fall to zero at 30, 40, or even 50 ft. as would be expected if all spread within 100 ft. of the source tree were due to primary root-graft spread. Figure 27 illustrates somewhat comparable data obtained in West Virginia by the oak wilt control personnel. It would seem that those portions of the disease distribution curves which lie beyond 30, 40, and certainly those beyond 50 ft. from the assumed source, represent the results of local overland spread for comparatively short distances, rather than the consequence of root-graft spread.

In Figure 28 the curve based on Boyce's oak wilt data, curve a, is compared with a curve b, drawn from data taken by Wolfenbarger and Jones (1943), on the incidence of crotch-feeding by scolytid beetles on twigs of elm trees standing at different distances from the point of insect

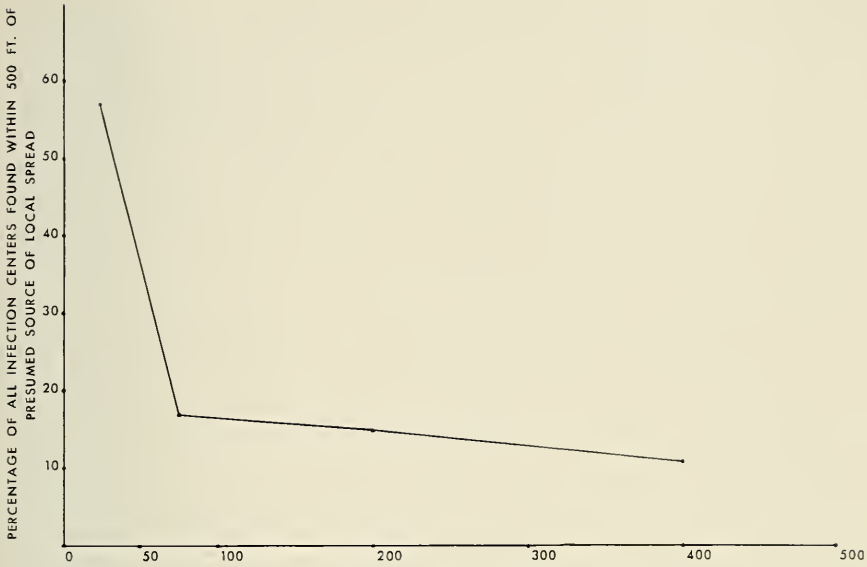


FIGURE 27. The numbers of wilting oaks found within designated distance classes from presumed sources of spread in West Virginia (Gillespie and Craig, 1957).

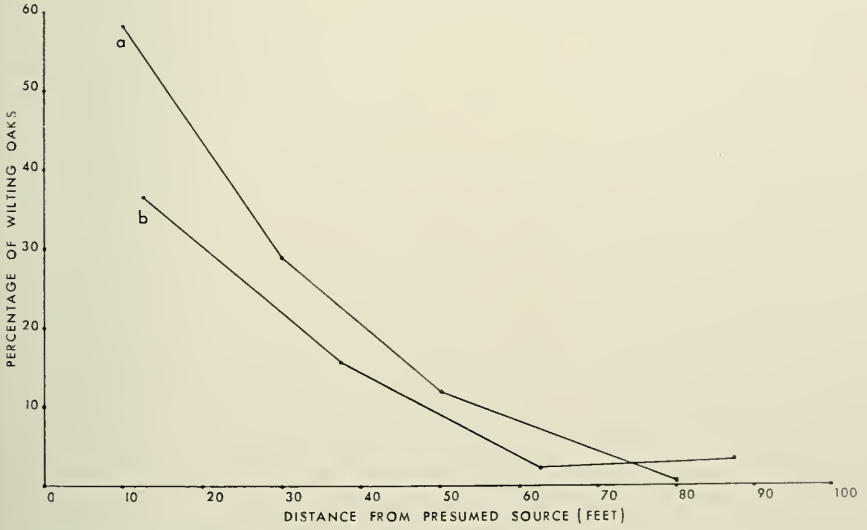


FIGURE 28. Comparison between percentage of all wilted oaks (curve a) found within designated distance classes from presumed source of local spread in North Carolina (Boyce, 1957) with the numbers of cases of crotch feeding due to *Scolytus multistriatus* (curve b) found within designated distance clsases from known points of beetle emergence (Wolfenbarger and Jones, 1943).

dispersal. The similarity of these curves, and the fact that the oak wilt curve does not fall to zero at the presumed limit of primary root-graft spread, suggests that in the Appalachians we are dealing, at least in part, with an air-borne agency of local disease spread. Such agencies may, but need not be, the same as those responsible for long-distance spread. One practical point to be derived from this discussion is the strong suggestion that a program which checks overland spread effectively may be expected to bring about some reduction in local spread.

LONG-DISTANCE SPREAD

Although oak wilt had been recognized and studied for more than ten years, the method of long-distance spread overland was not known until the summer of 1952 when Leach, True, and Dorsey (1952) described and interpreted the function of the pressure cushions that form beneath the bark and crack it to form a lenticular cavity in which the fungus mats develop. These authors pointed out that insects, principally nitidulids, were attracted to these cavities by the characteristic odor of the fungus and they demonstrated experimentally that the insects transported endoconidia of opposite compatibility type from mat to mat, thus serving as agents of spermatization for this heterothallic fungus. These workers also predicted that nitidulids, since they also visited wounds in the bark of healthy trees, would be found to be important vectors of the disease and the principal agents of overland and long-distance spread. The accuracy of this prediction was proved experimentally by Dorsey, Jewell, Leach, and True. (1953) in West Virginia and by Norris (1953) in Iowa. Further confirmation was presented in later years by Himelick, Curl, and Zuckerman (1954), Jewell (1954), McMullen, Drake, Shoenfelt, and Kuntz (1955), and Thompson, Hadley, and Jeffrey (1955).

The importance of these insects as vectors of oak wilt has been reviewed by Dorsey and Leach (1956) and by Jewell (1956).

It was observed that perithecia were frequently formed in abundance on insect-infested mats, while they were never observed on mats that developed below uncracked bark. Since it had been shown by Bretz (1952 c) that the fungus is heterothallic and by Hepting, Toole, and Boyce (1952 a) that perithecia are formed following spermatization with endoconidia of the opposite compatibility type, it seemed probable that the insects might be acting as agents of spermatization, transporting endoconidia from type A mycelium to spermatize mats of compatibility type B or *vice versa* (Leach, True, and Dorsey, 1952) as soon as the cracking of the bark permitted them to reach the fungus mats.

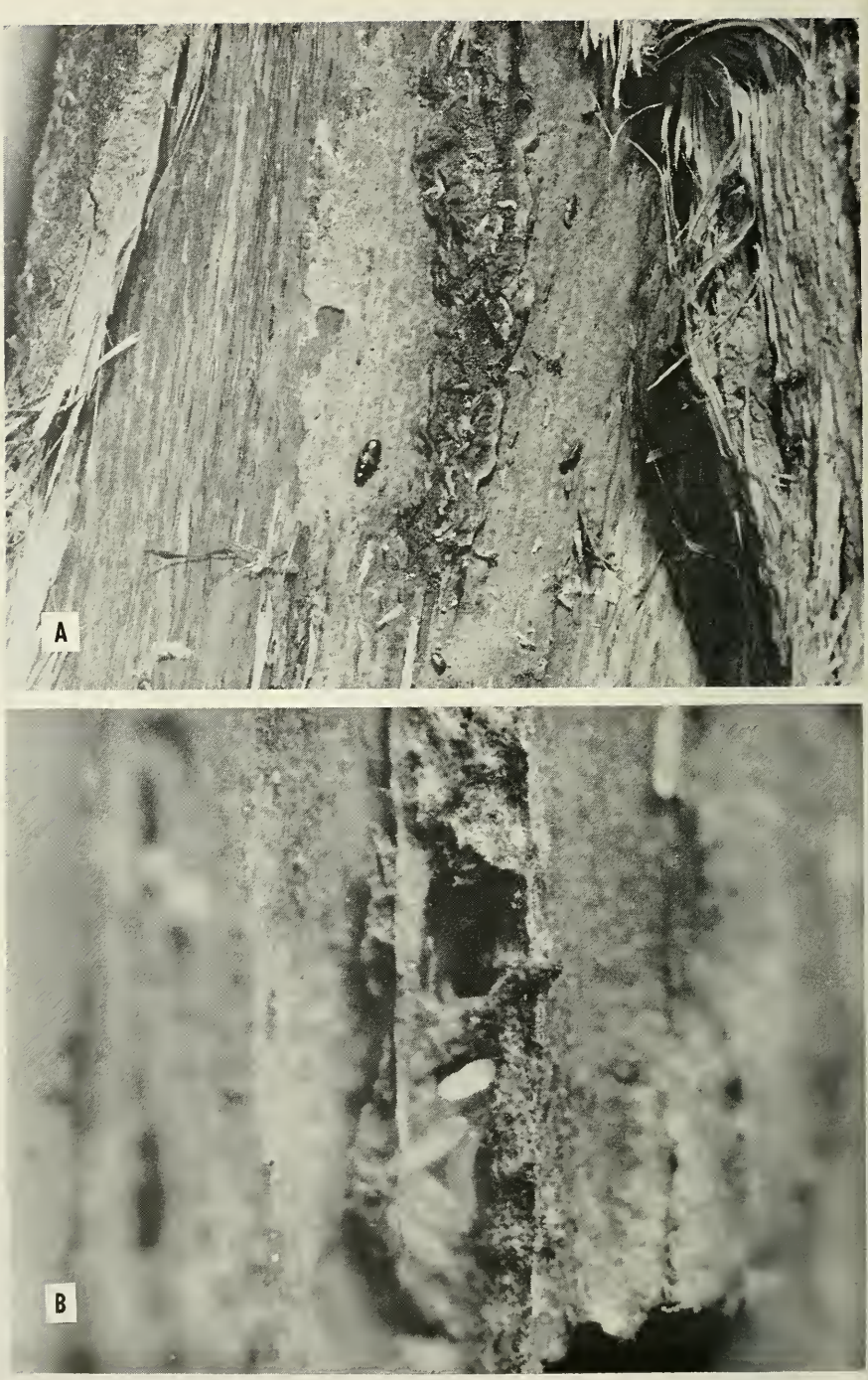
Barnett, Staley, and True (1952) had shown that the mycelial mats occurring beneath the cracked bark are ready for spermatization, and would produce perithecia within a few days when conidia of the opposite compatibility type were placed on them.

Leach, True, and Dorsey (1952) tested experimentally the hypothesis that the nitidulid beetles were agents of spermatization. Several blocks of wood or bark supporting mycelial mats were collected from an isolated tree killed by wilt in 1951. Only compatibility type A of *Ceratocystis fagacearum* had been cultured from this tree, and no perithecia had been found on it. These blocks were placed in a moist chamber and several nitidulid beetles, collected from the same tree, were allowed to crawl over a Petri dish culture of the B compatibility type, and placed in the moist chamber on the A-type fungus mats. Three days later, perithecia appeared on the mats. No perithecia were formed on mats held in a similar moist chamber, but not exposed to the insects.

As a further test (Leach, True, and Dorsey, 1952), beetles collected from a tree from which only the B compatibility type had been isolated were placed in a moist chamber with mats from the A tree. Again, perithecia appeared in three days. A third test was made by placing mats from the A and B trees in the same moist chamber with insects collected from the A tree. Here again, perithecia appeared on the A mats. No perithecia were observed on the B mats, but they were somewhat older than the A mats and may have been beyond the best stage for effective spermatization.

These experiments clearly showed that the nitidulid beetles can act as agents of spermatization, transferring endoconidia to and from mats of the two compatibility types. The fact that nitidulid beetles are actively attracted to these fungus mats may be easily demonstrated by removing a section of bark from above a mat, collecting all the beetles present, and then replacing the bark. On the following day, the mat will again be populated with beetles. If these are removed, a new supply will appear overnight as long as conditions are favorable. The activity of the nitidulids and their attraction to fungus mats have also been emphasized in experiments by Boyce (1954 a) who showed that liberated, marked, beetles infested fungus mats 500 feet from the point of liberation. Morris, Thompson, Hadley, and Davis (1955) showed that nitidulid beetles frequently moved to fungus mats from other situations, but tended to remain on mats until these dried out or were consumed.

The beetles not only feed on the mats, but they also breed there (Figure 29). Eggs are deposited on the mats, and within a few days the mats may become heavily populated with nitidulid larvae (Dorsey and Leach, 1956). The mycelial mats, as well as the fleshy pressure


A
B

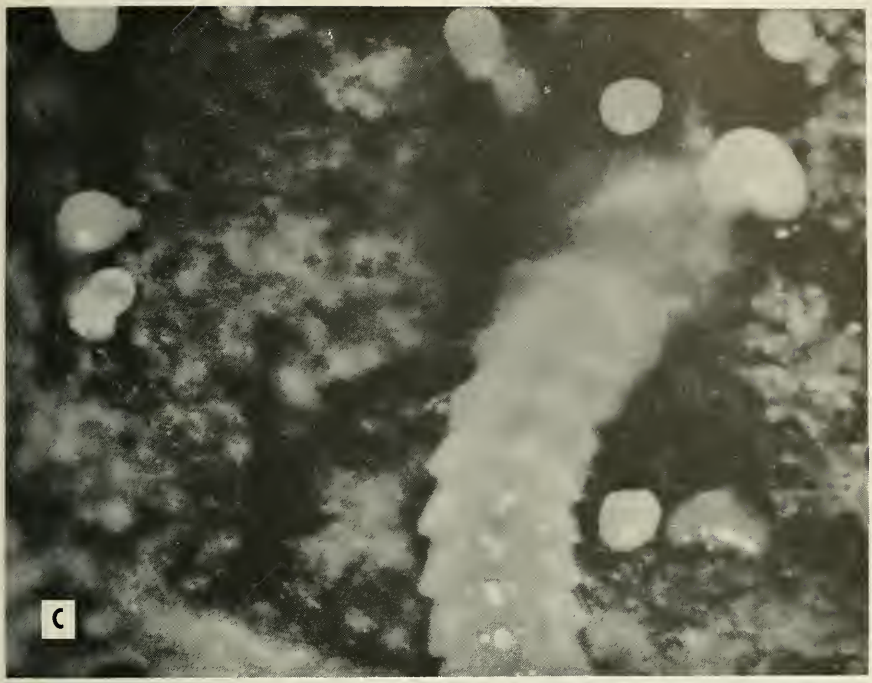


FIGURE 29. Adults, eggs, and larvae of nitidulid beetles on the mats of the oak wilt fungus. A. Adults and larvae exposed by removing the bark from over a mat. B. Nitidulid eggs as deposited on a fungus mat. C. A nitidulid larva feeding among the ascospores of the pathogen. Each glistening white mass constitutes the ascospores exuded from a single perithecium. The larvae, when mature, drop to the ground and pupate in the duff.

cushions may be overrun by the larvae of nitidulids, and other insects (including several species of Diptera and a number of predacious Coleoptera and Hemiptera).

Since several species of insects which inhabit the mats come into intimate contact with both endoconidia and ascospores (Dorsey and Leach, 1956) these are all potential vectors of the disease if they later visit healthy, susceptible trees under conditions which make infection and disease development possible. The nitidulids are known as sap-feeding beetles and are readily attracted to fresh wounds which expose the moist sapwood, so they were early considered likely vectors of oak wilt, probably carrying the spores from the oak wilt mats to fresh, sappy wounds in healthy trees. For this reason they were given special attention in early disease-transmission studies in West Virginia.

The first experiments with nitidulids, made in the summer of 1952, gave negative results, although several different types of inoculations were made. Since it was recognized that, for successful inoculation, the

nitidulids would be dependent upon the pre-existence of wounds, a search was made for naturally occurring wounds on oaks available for inoculation. Two of several old, bleeding wounds observed on a few large red oaks (Figure 30) contained one or more nitidulids when first observed. Some of these old bleeding wounds were subsequently inoculated by caging artificially contaminated insects upon the wound areas. Nitidulids which had been allowed to crawl over sporulating cultures of *C. fagacearum* in Petri dishes were released on other bleeding wounds. Later such wounds were inoculated also by spraying with a suspension of conidia and ascospores. None of these trees became diseased. Recent studies by other investigators (Cole and Fergus, 1956) have substantiated and explained these early negative results with old wounds.

Several other trees were inoculated by placing five to ten contaminated beetles in a 1 x 4-inch plastic capsule fastened over a small chisel-cut in the bark. Although the capsules were ventilated with numerous pin punctures, the beetles apparently did not find the environment suitable and soon died. None of the trees so inoculated became diseased.

FIGURE 30. A bleeding wound at the base of a large oak tree. Such wounds are not satisfactory infection courts. Fresh wounds are necessary.

In the spring of 1953 additional trees were inoculated (Dorsey, Jewell, Leach, and True, 1953) using four species of nitidulids (*Colopterus semitectus* (Say), *Glischrochilus sanguinolentus* (Ol.), *G. confluentus* (Say), and *Carpophilus lugubris* Murr. The inoculation procedures used were essentially like those of the previous year (Figure 31) except that in making the chisel cut, an effort was made to separate the inner bark from the sapwood at the cambium, to provide the moist, protected type of wound found naturally attractive to these insects. In most cases, larger screen cages were used, covering large, blaze-type wounds. To contaminate the insects they were placed from one to two hours in Petri dishes containing cultures of *Ceratocystis fagacearum* which were producing both ascospores and endoconidia. Insects thus contaminated were introduced into the cages. Some trees were inoculated at two separate points. Approximately twenty beetles were caged on each tree. The inoculated trees were observed at weekly intervals, and on June 12, five of six trees inoculated May 6 showed symptoms of wilt. Positive identification of the oak wilt fungus was made June 16 in cultures made from samples taken from the symptom areas. A few additional trees inoculated later in May developed oak wilt, but many remained healthy. Extremely dry weather prevailed during June and most of the remaining summer months, and this may have been unfavorable for infection. It was observed that the wounds made in June or later dried rapidly and became unfavorable habitats for the insects. Dorsey and Leach (1956) found that the period of greatest attractiveness of axe cuts and bruise wounds to wound-inhibiting insects (listed on page 74) was during May and June in the case of oaks, and from

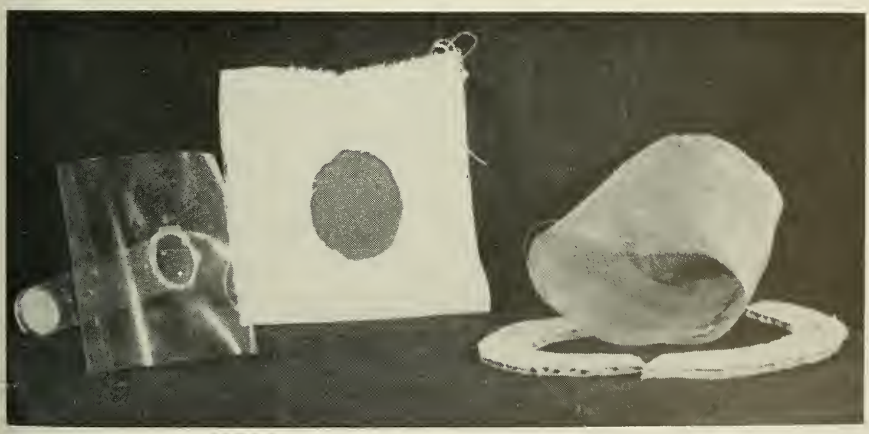


FIGURE 31. Three types of cages used in transmission experiments with nitidulids. The all-celluloid cage on the left was not as satisfactory as the other two (Jewell, 1956).

COLEOPTERA COLLECTED FROM FRESH WOUNDS IN OAK TREES IN WEST VIRGINIA DURING 1953-1954

NITIDULIDAE

Carpophilus lugubris Murr.
Carpophilus sp.
Colopterus semitectus (Say)
Glischrochilus fasciatus (Ol.)
G. sanguinolentus (Ol.)
Colopterus truncatus (Rand)
C. morio (Er.)
Cryptarcha ample. Er.
Stelidota geminata (Say)
Lobiopa undulata (Say)
Epuraea sp.

OSTOMIDAE

Tenebriodes sp.

HISTERIDAE

Platystoma sp.
Hister sp.

CUCUJIDAE

Silvanus sp.

STAPHYLINIDAE

Philonthus sp.

BRENTIDAE

Arrhenodes minuta Drury

CURCULIONIDAE

Conotrachelus anaglypticus (Say)

February through July in the case of maples. Wounds freshly made in oak during May and June remained attractive to nitidulids and to other insects, found in smaller numbers, for about three weeks, or until the exposed sapwood became dry beneath the loosened bark.

Although nitidulids may spread oak wilt during dry weather, wet weather (Cole and Fergus, 1956; Drake, Kuntz and Riker, 1957), and the spring season (Jeffrey, 1953; Craighead, Morris and Nelson, 1953; Kuntz and Drake, 1957) seem to favor successful inoculation of cambium-depth wounds such as those that especially attract nitidulids.

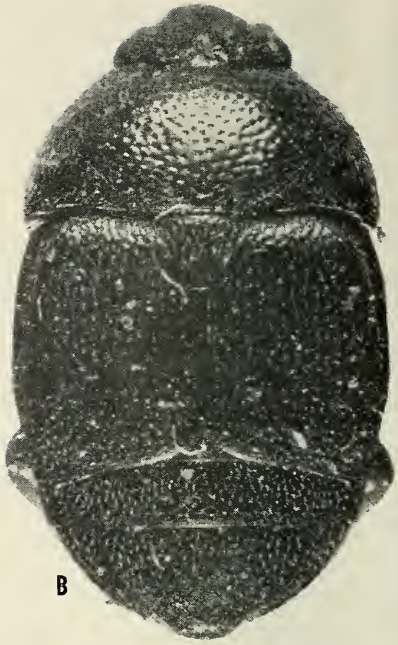
At about the same time that the successful inoculations were made, similar wounds were made on other trees and left uncaged. Within a few days these were visited by numerous insects, most of which were

nitidulids of the same species used in the inoculations (Figure 32). In fact, most of the nitidulids used in subsequent inoculation experiments were caught in such wounds. Within three days after the wounds were made, they were found to contain nitidulid larvae of at least three species. Often as many as 50 adult nitidulids were collected at one time from a single wound. These observations showed that the insects used in the experiments normally visit wounded, non-diseased trees under conditions suitable for the transmission of oak wilt. These insects, as previously shown, also regularly visit the mycelial mats and become contaminated with the spores of the fungus. The production of the disease experimentally with these same species of insects seems to justify the conclusion that they are important vectors of the disease in nature (Dorsey, Jewell, Leach, and True, 1953; Jewell, 1956).

It is recognized that there may be additional vectors, as reported by other workers (Craighead and Morris, 1952; Griswold, 1953; Griswold and Neiswander, 1953; Griswold and Bart, 1954; Stambaugh, Fergus, Craighead and Thompson, 1955; Curl, 1956; Himelick and Curl, 1958, Buchanan, 1957, 1958) and suggested by the long list of insects found associated with oak wilt mats (Table 8), but the feeding habits of the nitidulids and their constant association with the disease point to the conclusion that these insects are of primary importance. The formation of mats beneath the bark with the superimposed pressure cushions that crack and lift the bark, the odor attractive to insects, the method of spermatization and the conditions of ascospore formation (Figure 15) all constitute a highly developed adaptation with mutual benefits to insect and fungus. Such a complex adaption could evolve only if it had a strong survival value in perpetuating the fungus and in providing food and shelter for the insect. Even though other insects may sometimes transmit the disease, and the nitidulids may survive in the absence of oak wilt mats, those insects that regularly visit oak wilt mats and also inhabit suitable infection courts meet all the requirements of an effective vector.

Under West Virginia conditions nitidulids have been regularly collected every month of the year. The species found varied with the season, but some nitidulids were present and active at all times of the year except when the mean weekly temperature dropped below 32° F. Maximum numbers of nitidulids occur in the early spring and in the fall when mean weekly temperatures approximate 50° F. (Dorsey and Leach, 1956). These insects normally do not penetrate uninjured bark. They mainly inhabit wounds made by other agencies. Craighead and Morris (1952) have pointed out that nitidulids are relatively inefficient vectors when compared with such vector insects as the smaller European


A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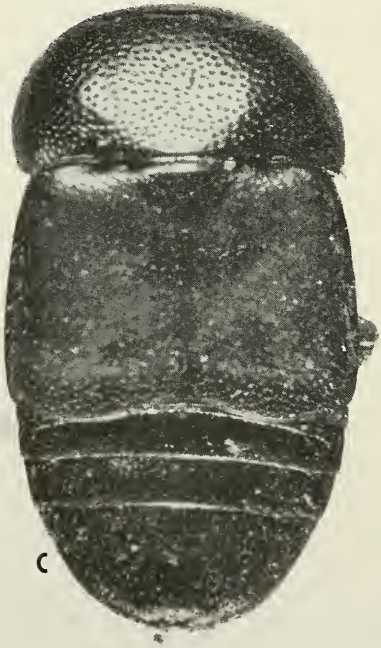
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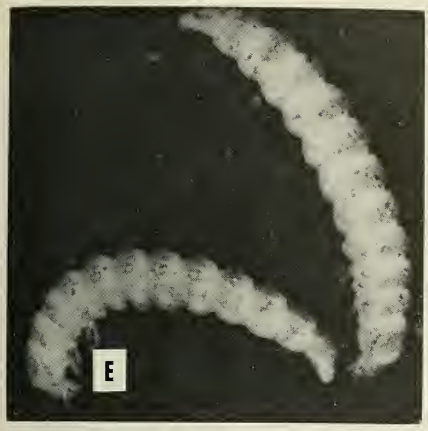

FIGURE 32. Nitidulid beetles, the most important vectors of oak wilt. A. *Glischrochilus sanguinolentus*. B. *Colopterus morio*. C. *Colopterus semitectus*. D. A specimen of *Colopterus semitectus* mounted and photographed to show the flat, thin, bodies of these beetles that permit them to enter small cracks and to move about beneath loosened bark. E. Two nitidulid larvae, species not determined. A magnified 15x. B magnified 19x. C and D magnified 23x. E magnified 6x.

elm bark beetle which is chiefly responsible for spreading the Dutch elm disease in the United States (Collins, Buchanan, Whitten, and Hoffman, 1936).

The West Virginia experiments showing that nitidulid beetles are capable of transmitting oak wilt were substantiated by similar experiments reported at the same time from Iowa by Norris (1953), who used four species of nitidulids, (*Epurea corticina, Glischrochilus fasciatus, G. quadrisignatus* and *G. sanguinolentus*). Of these only *G. sanguinalentus* was included in the West Virginia studies.

In West Virginia all of the several species of Nitidulidae listed on this page have been collected from fungus mats on oak wilt trees. Although experimental evidence is not available for all species, it is believed that any of these nitidulid beetles inhabiting oak wilt mats can

PRINCIPAL SPECIES OF NITIDULIDAE FOUND ASSOCIATED WITH OAK WILT-INFECTED TREES IN WEST VIRGINIA

Glischrochilus quadrisignatus (Say)
G. fasciatus (Ol.)
G. sanguinolentus (Ol.)
G. confluentus (Say)
Colopterus semitectus (Say)
C. morio (Er.)
Carpophilus lugubris Murr.
Carpophilus sp.
Epuraea spp.
Prometopia sexmaculata (Say)
Cryptarcha ample (Er.)
Stelidota geminata (Say)

serve as a vector of the disease. Because of their feeding and breeding habits, their relatively high reproductive potential, their habits of flight and dispersal, their quick response to stimuli and because some species are present during every season of the year, the nitidulids would be expected to make effective (if not efficient) vectors of the oak wilt disease. The importance of nitidulids as oak wilt vectors has been confirmed more recently by workers in Illinois (Himelick, Curl, and Zuckerman, 1954), Pennsylvania (Thompson, Hadley, and Jeffrey, 1955) and Wisconsin (McMullen, Drake, Shenefelt, and Kuntz, 1955).

Some nitidulid beetles come into close physical contact with both endoconidia and ascospores in nature on the fungus mats of *Ceratocystis fagacearum*. Transmission of wilt could, therefore, occur naturally (as well as in the case of artificially contaminated beetles) as the result of spores adhering mechanically to the external parts of their bodies. Since these insects not only crawl over the fungus mats, but also feed upon them, there was need to learn whether ingested spores could pass through their intestinal tract and be excreted in the feces in a viable condition. Experiments proving that viable endoconidia and ascospores are present in the feces of nitidulids have been reported by Jewell (1954, 1956). Microscopic examination of fecal pellets freshly excreted by beetles previously fed on cultures bearing endoconidia revealed numerous spores resembling the endoconidia of *Ceratocystis fagacearum* (Figure 33), but efforts to germinate the spores and isolate the fungus in pure culture failed. When the fecal pellets were plated on various media (including water agar), the spores were quickly overrun by bacteria and yeasts. This difficulty was overcome by the device of spermatizing[12] cultures belonging to both compatibility types with the fecal pellets and watching for the development of fertile perithecia. Their appearance was considered evidence that the mycelium had been spermatized by viable conidia of *C. fagacearum* belonging to the opposite compatibility type, contained within the fecal pellets.

In these experiments, two species of nitidulids were allowed to feed for one week on Petri dish cultures of type A *Ceratocystis fagacearum*. The beetles were then removed and glued upside down on sterile glass slides in sterile Petri dishes. The beetles, inverted and immobilized, were then brushed several times with 95 per cent alcohol to eliminate or reduce viable surface contamination. The beetles were closely observed under a stereoscopic microscope, and as defecation occurred, the pellets were removed with a cool, flame-sterilized needle. The pellets were

[12]The spermatization technique involves the application of material presumed to contain spores or mycelium of *C. fagacearum* to receptive mycelium of either compatibility group in an attempt to induce the production of perithecia in the A or B test cultures.

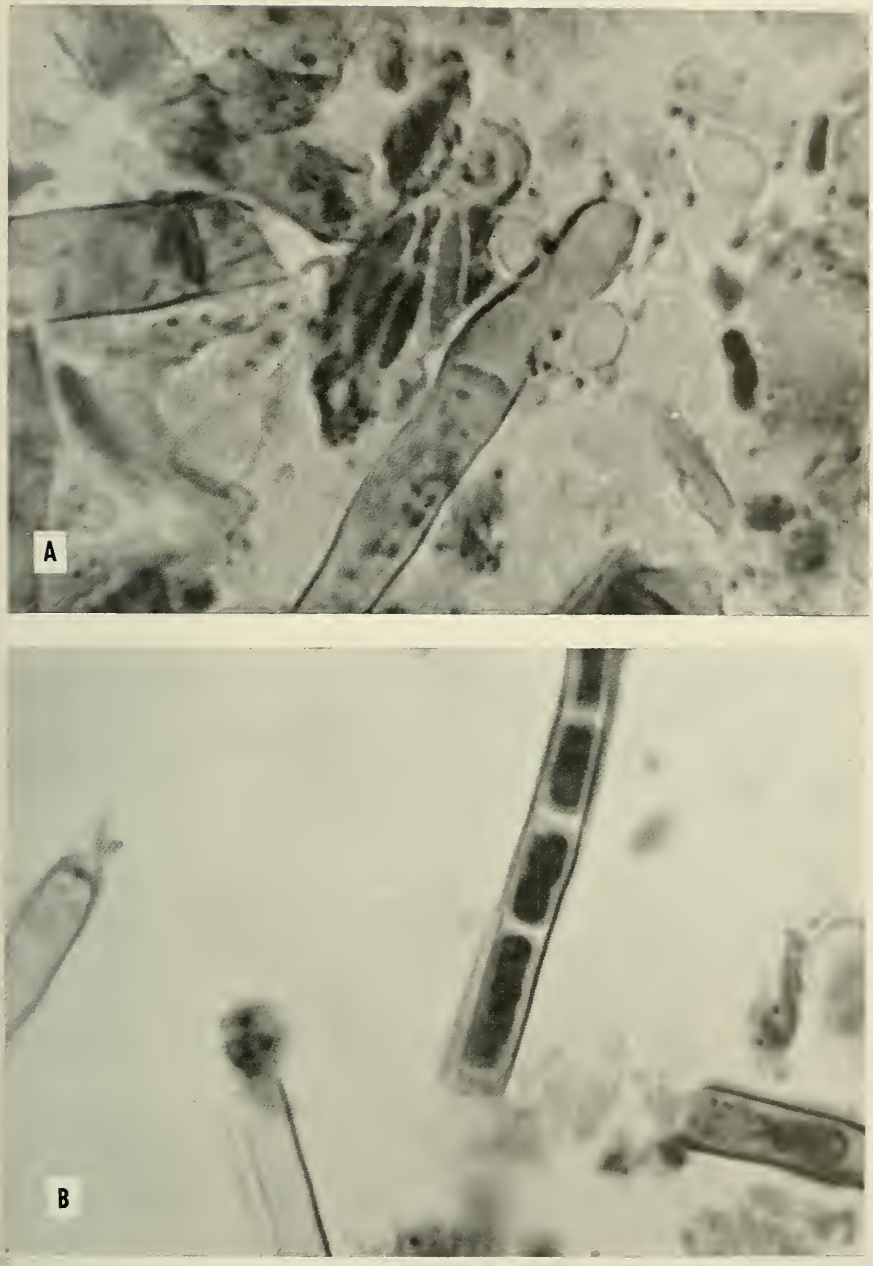

FIGURE 33. Photomicrograph showing ascospores (A) and endoconidia (B) in the fecal pellets of nitidulid beetles. Such spores are usually viable and uninjured after passage through the insect and may serve as inoculum or serve in spermatization (Jewell, 1956).

crushed in a drop of sterile water on top of 7-day-old colonies of type B mycelium, using similar colonies of type A as controls. The spermatized cultures were incubated for three days at 20° C. and then examined for the presence of fertile perithecia. A total of 75 colonies were spermatized with the feces from 45 nitidulid beetles; 66 colonies were type B and 9 were Type A. Sixty-seven per cent of the type B colonies produced fertile perithecia (from 2 to 30 per colony), while none of the type A colonies gave positive results.

These results demonstrated clearly that the conidia and/or mycelial fragments of the oak wilt pathogen pass through the intestinal tract of the nitidulid beetles in a viable condition, capable of spermatizing the mycelium of the opposite compatibility type of *Ceratocystis fagacearum*. This technique has since been used successfully by other workers in demonstrating the presence of the pathogen in or on the bodies of bark beetles emerging from infected trees (Stambaugh, Fergus, Craighead, and Thompson, 1955), and in showing that the conidia and ascospores of the pathogen can survive winter temperatures in or on the bodies of nitidulids artificially maintained at these temperatures for as long as 121 and 151 days, respectively (Stambaugh and Fergus, 1956).

Other Possible Insect Vectors of Oak Wilt. Although it is believed that the nitidulids are the most important vectors of oak wilt, numerous other insects have been found associated with the fungus mats, and perhaps several of these may transmit the disease. It seems probable that insects not associated with fungus mats would seldom become sufficiently contaminated to transmit the disease.

As has been pointed out by Leach (1940), "In order to be an effective vector of a plant disease, an insect must visit, with a reasonable degree of regularity, both diseased and healthy plants. The mere association of an insect with the diseased plants or the presence of inoculum on or in the insect's body is not sufficient justification for concluding that the insect is a vector of the disease. Unless the insect bearing the inoculum visits a healthy plant under conditions conducive to inoculation and infection, it may be of no significance in the transmission of the disease."

Among the possible vectors associated with the fungus mats are several species of Diptera, including *Drosophila melanogaster* Meig. Bart and Griswold (1953) have shown that this insect ingests the spores of the pathogen and that these can be recovered in a viable condition from the crop and from the feces. If it is shown that in some oak wilt areas where fungus mats are formed this insect visits the fungus mats in large numbers and also consistently inhabits fresh wounds on healthy trees, there would be ample justification for suspecting it to be a vector of

oak wilt. Recently the existence of wild types of *Drosophila,* native to forest sites, has been pointed out (Dorsey and Carson, 1956). Griswold (1958) has shown that several of these are capable of transmitting oak wilt.

It has been shown that the oak bark beetle *Pseudopityophthorus pruinosus* Eichh. breeds in diseased trees although it is seldom found on oak wilt mats. These beetles are, nevertheless, sometimes contaminated with *Ceratocystis fagacearum* upon emergence (Stambaugh, Fergus, Craighead, and Thompson, 1953). Under experimental conditions, artificially contaminated oak bark beetles have, in a small percentage of cases, inoculated healthy oaks upon which they were caged in large numbers to feed (Griswold and Bart, 1954; Buchanan, 1958). Supporting field evidence upon which to evaluate their importance in transmitting oak wilt is at present lacking.

Curl (1956) has shown that certain species of Collembola are commonly found on oak wilt mats, and when specimens were artificially contaminated and promptly caged on 20 freshly wounded oak seedlings, 7 of the 20 became infected. No infection occurred when Collembola were held 16 hours before being placed on the wounds. This may indicate that the spores remained viable for less than 16 hours on the insects. In view of this, and because the Collembola are dependent upon wind for long-distance dissemination, it seems unlikely that they would be very effective vectors in nature. Possibly they could serve as vectors for local spread under favorable weather conditions.

Curl (1955 a) also listed 40 species of insects (Table 8) including representatives of 19 families collected from oak wilt mats, pointing out that all were potential vectors. Dorsey and Leach (1956) reported specimens representing 31 families of Coleoptera along with a few miscellaneous specimens of Hymenoptera, Diptera, Orthoptera and mites, all collected or reared from oak wilt mats (Table 8).[13] It was recognized that all of these would become contaminated with spores of *Ceratocystis fagacearum,* but it was pointed out that of all the insects found, the Nitidulidae were most numerous and most consistently present, and that because of their life habits they are likely to be the most effective vectors of oak wilt.

Much work will be required before the relative importance of the different species of insects can be determined. Their importance and effectiveness may vary in different sections of the country and in different seasons.

[13]Table 8 includes in column 2 additional insects collected in association with oak wilt mats during the 1957 summer survey in West Virginia. These included two additional families of Coleoptera; the Coccinellidae and the Byturidae not included in Dorsey and Leach's earlier list (column 1). Insects belonging to the orders Hemiptera, Thysanura Collembola, and Psocoptera were added in 1957.

TABLE 8. PRINCIPAL INSECTS ASSOCIATED WITH FUNGUS MATS

INSECTS	OAK WILT				*Hypoxylon* SPP.
	W. VA. 1952-53	W. VA. 1957	PA.* 1950	ILL.** 1955	W. VA. 1957
Coleoptera					
Nitidulidae	+	+	+	+	+
Tenebrionidae	+				+
Cerambycidae	+	+			+
Coccinellidae		+			+
Ostomatidae	+	+			+
Rhizophagidae	+		+	+	
Carabidae	+			+	+
Colydiidae	+		+	+	
Lycidae					+
Mycetophagidae	+			+	
Sandalidae					+
Orthoperidae	+				
Scolytidae	+		+	+	
Brentidae	+		+	+	+
Cucujidae	+		+	+	+
Platyplodidae	+	+			
Dascillidae	+				
Pythidae	+				
Cleridae	+				+
Mordellidae	+				
Buprestidae	+				
Staphylinidae	+		+	+	+
Pselaphidae	+				
Chrysomelidae	+				+
Curculionidae	+		+	+	+
Byturidae		+			+
Cryptophagidae					
Scarabaeidae			+	+	+
Serropalpidae	+				
Derodontidae				+	
Histeridae	+	+	+	+	+
Melandryidae					+
Lathriidae	+				
Throscidae	+				
Cisidae	+				
Leiodidae	+				
Anobiidae	+				
Monotomidae	+		+		
Endomychidae			+	+	
Elateridae	+		+	+	+
Rysodidae					+
Diptera					
Drosophilidae	+		+		
Syrphidae		+			
Ceratopogonidae			+		
Trichoceridae				+	
Stratiomyidae			+		
Hemiptera					
Pentatomidae		+			
Aradidae		+			
Anthocoridae		+		+	

Table 8. Continued

Insects	Oak Wilt				*Hypoxylon* spp.
	W. Va. 1952-53	W. Va. 1957	Pa.* 1950	Ill.** 1955	W. Va. 1957
Thysanura					
Campodeidae		+			
Psocoptera					
Psocidae		+			
Homoptera					
Fulgoridae		+			
Hymenoptera					
Formicidae	+	+			
Orthoptera					
Gryllidae		+			
Blattidae	+	+			
Collembola		+		+	
Acarina	+				

*Craighead, 1954.
**Curl, 1955a.

It is interesting to note from Table 8 that at least 15 families of coleopterous insects found on the fruiting stromata of *Hypoxylon* spp. during the 1957 survey have also been observed on oak wilt mats. Possibly some of these insects may at times carry the spores of both fungi.

Possible Vector Agencies Other Than Insects. Curl (1955 b) was able artificially to blow spores of the oak wilt fungus out through bark cracks associated with the subcortical fungus mats of diseased trees. He postulated that wind storms might cause endoconidia to become air-borne and so to be disseminated to suitable infection courts. His findings have not substantially changed the earlier conclusions of most workers that oak wilt is seldom if ever spread through the dissemination of wind-borne spores. Wind currents seem more likely to assist in disease spread by influencing the movements of spore-laden insect vectors (Thompson, 1951).

Several workers have suggested that birds might serve as vectors of wilt, becoming contaminated while feeding on insects inhabiting the mats of the pathogen. Observations, however, have revealed no evidence that birds visit the mats with any degree of frequency, and all efforts to isolate *Ceratocystis fagacearum* from the beaks of birds that have fed on oak wilt mats have failed (Tiffany, Gilman, and Murphy, 1955).

In the midwest, squirrels have been observed to feed upon the fungus mats and have been suspected of transmitting the disease (Himelick, Schein, and Curl, 1953). Transmission by squirrels under artificial conditions has been reported (Himelick and Curl, 1955), but there is little information to indicate that these animals are important vectors of oak wilt in nature.

Jones and Bretz (1955) tested the efficacy of the ax, pole pruner, saw and pole saw as agencies of disease transmission. Each tool was tested in two ways. The cutting edge was moistened with a suspension of endoconidia and of ascospores just prior to cutting into a healthy test tree. The use of these tools freshly inoculated with spore suspension caused successful infection and disease transmission in many instances. When attempts were made to contaminate the tools by cutting into the infected tissues of diseased trees, and to transmit the disease by using these tools immediately afterward to cut into the wood of healthy trees, none of the healthy trees so injured developed oak wilt.

Acorns from an infected oak have not yielded the oak wilt pathogen (Bretz and Buchanan, 1957).

Present evidence justifies the conclusion that insect transmission is the principal means of overland spread of oak wilt and that nitidulids are the most important vectors. If study should show abundant sporulation of *C. fagacearum* within the galleries or feeding cavities of such oak bark beetles as feed upon the live twigs of healthy oaks, then the importance now attributed to insects which inhabit subcortical oak wilt mats might have to be shared. Until such time, the nitidulid beetles must be considered most important among insect vectors, especially in regions where fungus mats provide the chief source of inoculum.

Oak Wilt Control

The control of tree diseases is an expensive procedure which should be thoroughly justified before it is undertaken. The tree crop threatened must be of acknowledged importance to the region. The seriousness of the expected losses must be considered, and the chances of successful control should be evaluated.

When apparently threatening new diseases first appear, neither the seriousness of resultant losses nor the chances of controlling the disease can be immediately known. Those responsible for initiating survey and control work frequently must base their decision upon the best information available, even though it be inadequate. The first decision may rest on the apparent danger involved if attempted control should be longer delayed. With the passing of time, and with increased information about the disease, the original policy is justified, modified, or given up.

Most states in which oak wilt has been found have already made their first decision in regard to attempting control, and they are waiting to see whether this decision will prove to be right. Generally speaking, states on the eastern edge of the known oak wilt area (Pennsylvania,

Maryland, Virginia, West Virginia, Kentucky, Tennessee, and North Carolina) have undertaken active control programs, usually with federal assistance. Some states farther west are confining their work chiefly to surveys, while others are trying different controls on a limited experimental basis. Some midwestern states have undertaken no control activities. Surveys in New York, New Jersey, and a few other states in the East have revealed no oak wilt.

Presumably the middlewestern states will have the benefit of eastern control experience when the time comes for re-examination of their first policy, provided that the disease has not, by that time, become so intensified there as to make attempted control no longer justifiable. Citizens of the eastern states who may become skeptical of the need for annually recurring expenditures to combat oak wilt at home will watch with interest its uncontrolled development in several midwestern states. Wherever control programs are successful, the need for them will not be locally evident. Some areas of uncontrolled disease development are needed for comparison.

It has now become clear that oak wilt spreads and intensifies its losses rather slowly in Wisconsin (Anderson and Skilling, 1955), although the present rate of loss seems ultimately to threaten the complete destruction of oaks within the survey region. It is evident, however, that the establishment of experimental areas within the Appalachian region, in which the disease might be left for a time unchecked, would serve as a better guide in regard to the need for and value of a control program for oak wilt in the East. Recently the federal government has undertaken a plot-comparison study of the need for and effectiveness of oak wilt control measures as practiced in different states of the Appalachian oak wilt infection area.

POSSIBLE PATTERNS OF EXPENDITURE FOR OAK WILT CONTROL

Those responsible for deciding to undertake a control program in the oak wilt states of the Appalachian region had to determine whether this program should be aimed, like the earlier federal program of Dutch elm disease eradication, at the complete elimination of the disease in the immediate or very near future, or whether it should aim at disease suppression, with eradication as an ultimate desirable (though perhaps unattainable) objective.

Several factors govern such a decision. Few forest tree diseases have ever been eradicated, and these were quite localized at the time of their discovery (the European larch canker disease in Massachusetts). The characteristics of the pathogen, the host, and the possible vectors would

go far toward determining whether eradication or suppression were the more biologically sound approach. The availability of money and of manpower capable of doing the work may, in the last analysis, determine what is done.

A few years after the Dutch elm disease was found established in the East (1931) the availability of federal and state relief funds to hire the unemployed manpower, then abundant, suggested that it would be a good gamble to try at once for disease eradication even at a tremendous immediate expenditure. Even the seemingly exorbitant cost of a short-term successful eradication program would be less than losses to the states and affected land owners from the death of their elms, and less than the cost of an indefinitely long program of disease suppression. The attempt to eradicate the Dutch elm disease failed after approximately $10 million had been spent in the effort (American Forestry Association, 1938). Now, 25 years later, several states are undertaking long-term programs of the suppression type.

WEST VIRGINIA'S DECISION TO CARRY ON AN OAK WILT SUPPRESSION PROGRAM

West Virginia has chosen to undertake the control of oak wilt with a suppression-type program for several reasons: (1) the disease was already widespread within the State when it was first discovered; (2) the disease is present in neighboring states which may or may not attempt or continue to control it; (3) oak wilt may spread overland for such indefinitely long distances that surveys to locate infections coming from beyond our borders would still be required even if eradication could be achieved in West Virginia; (4) not enough is yet known about the disease, all possible sources of inoculum, the vectors, and how they serve to spread the disease, to enable us to set up an eradication-type program with any reasonable assurance of success; (5) West Virginians are already witnessing the successful operation of the white pine blister rust control program which is of the disease-suppression type; (6) financial support for control is so limited in any one year that only a suppression-type program can now be financed; and (7) sufficient manpower suitable for a seasonally limited eradication program is not available. Training and experience are required to do this work well, and the employment of large numbers of untrained men would involve critical inefficiency of operation.

Because we believe that in order to save ourselves from greater losses we must look forward to a continuing program of financial outlay for oak wilt suppression, we must consider what the cost of such an oper-

ation might be and weigh this against the value of the resources which the program is designed to protect.

If we think of this on the limited basis of oak stumpage values as reported for West Virginia by the U.S. Forest Service, Timber Resources Review, we find that the net volume of oak saw timber in West Virginia in 1953 was 7,972 million board feet (U. S. Forest Service, 1958). The net volume of all the oak growing stock was 39 million cords. If all this saw timber should be cut in a single year, and a stumpage price of $20 per thousand board feet be used in calculating its value, the oak saw timber standing in the State may be estimated as worth $159 million, retail, for lumber and dimension stock alone. Adding to this an allowance for young growth to increase the figure to $170 million and discounting this total 50 per cent (to reflect deferment of harvest and risk) would give a conservative wholesale value of $85 million.[14] This furnishes one measure of the value of the resource being protected by the control program. Since 1951, somewhat more than $200,000 has been spent to control oak wilt in West Virginia. This may be considered a small initial expenditure to protect such a valuable resource.

Another way to evaluate the repeated annual cost required for a sustained program of disease suppression is to compare the annual estimated cost of suppression with the estimated annual income from the crop protected. To date the highest annual cash outlay specifically designated for oak wilt control in West Virginia has been $65,000 (Gillespie and Craig, 1958). The Forest Industry Committee estimate of annual income to West Virginia from oak for the year 1954 amounted to nearly $20 million (see page 28). The maximum annual expenditure for oak wilt suppression has amounted to less than a half of one per cent of this estimated annual income from oak.

In our time the cost of protecting tree crops must be thought of in terms of disease and insect control, as well as protection from fire. A recent U. S. Forest Service report (Hepting and Jamison, 1958) shows that, on a nation-wide basis, insect damage alone now exceeds fire losses, and when all disease and decay losses are taken together, they exceed the growth and mortality losses due to insect pests.

White pine blister rust and oak wilt have both increased the cost of producing timber in West Virginia. The tree crops threatened by both are worth protecting, but the efficiency of suppression programs must always be scrutinized and new ways sought through research and good management to keep the annual costs low. We must face the increasing cost of forest protection, and we need to recognize that money

[14]Based on information furnished by Mr. Ellis Williams, Forest Economist, U.S. Forest Service, Washington, D.C., April 17, 1958.

expended on related research may be expected to increase the efficiency as well as the effectiveness of forest insect and forest disease control programs. The adoption of the deep girdle as the accepted method for processing oak wilt trees in lieu of cutting and burning them has reduced the average cost of ground procedures involved in treating diseased trees in West Virginia from $36 to $7 per tree. Before we discuss further the various means of treating oak wilt trees, let us consider how diseased trees may best be discovered.

SURVEYING FOR OAK WILT

The purpose of an oak wilt control survey, carried out as part of a disease-suppression program, is to find as many diseased trees as the time, money, and circumstances permit, to find all infection centers before they become large, and to do this so effectively that the disease does not spread from undiscovered infection centers to cause serious or regularly increasing losses. No matter how good the measures used for processing diseased trees, these can be made ineffectual by a survey which leaves too many unrecognized infection centers from which the disease may spread for a time undetected.

The generally conspicuous and reasonably distinctive symptoms of oak wilt, which make airplane survey for this disease possible, go far to offset the difficulties induced by the rough West Virginia terrain and the comparative inaccessibility of many of our forested areas. The air survey procedures recommended by Fowler (1951) have been used, with some modifications made necessary by the mountainous terrain. Contour flying of watersheds has replaced the grid-type of flight pattern found suitable for flat lands. In making these changes, the adherence to recommended altitudes and distances between flight lines has insured comparable coverage of our more difficult and dangerous mountainous areas even though it has meant that West Virginia flight costs are higher, and flight time required per hundred square miles of coverage has been somewhat greater than figures reported from neighboring states.

Even under the best conditions, it is not possible to find all oak wilt trees by air scouting. Air surveys which cover an area only once during the summer cannot be expected to locate trees that show symptoms after that survey. If the date of survey is delayed to minimize this difficulty, then many early-season trees may have lost so many of their leaves as to be easily missed. West Virginia experience has suggested that three to five judiciously spaced air coverages per summer are much more effective than one or two. Some few diseased trees shed their leaves so rapidly that even flights made at two- to three-week intervals may fail to locate them (Gillespie and Craig, 1958).

In any case, the ground crews which go in to examine, diagnose, and process the disease suspects located by the airplane spotters often find additional oak wilt trees on their way to or from the suspect trees. The location of disease suspects is marked on the map by a dot, and in the forest by the near presence of an unfurled roll of toilet paper thrown from the spotting plane. The finding of this field marker assures the ground crew that they have located one of the trees designated on the airplane observer crew's map. Oak wilt trees which they find lacking such a marker are usually considered new symptom trees or trees not seen by the plane's observer crew.

It is not remarkable that some diseased trees are missed from the air since oak wilt trees are not always large, dominant or codominant trees. Symptoms in trees with small, suppressed crowns can be easily missed from the air. The localized symptom areas which characterize the disease in larger trees of the white-oak group or the dark, dull brown discoloration symptomatic of oak wilt in scrub oak (*Q. ilicifolia* Wang.) are seen only with difficulty. Yet air scouting is an essential part of the program, and it seems that repeated air surveys together with foot scouting by the follow-up ground crews give reasonably satisfactory results. Under some conditions, however, even the best survey methods will fall far short of the mark. The effectiveness of symptom scouting for oak wilt is greatly reduced in areas where large numbers of oaks have died from other causes, such as the effects of fire, or repeatedly recurring droughts (Fergus and Ibberson, 1956; Gillespie, 1956; Tryon and True, 1958), or in regions where the inroads of the periodical cicada have been extremely severe, or when cases of late-season oak wilt infection have led to the production of premature autumn discoloration as the only symptom of the disease (Stambaugh and Nelson, 1956).

Recently it has been found possible to locate some established oak wilt infection centers in the absence of foliar symptoms by plotting the locations of groups of dead oaks from the air, even though none of the trees show current leaf symptoms (Gillespie and Craig, 1958). Oak wilt mats may be found on some of the more recently killed trees, or ground scouting may lead to the discovery of some trees with inconspicuous foliar symptoms. This procedure of plotting groups of dead trees as potential oak wilt centers cannot be used efficiently in areas where drought-associated oak mortality is common, where fire has killed many trees recently, or where serious injury has resulted from the prevalance of toxic industrial fumes.

Our admitted inability to see or recognize all oaks affected by oak wilt is one reason why we must be satisfied with a disease-suppression

program rather than one aimed at early complete eradication. In spite of the difficulties reported here, there is evidence that when finances have permitted an adequate job of scouting, the survey portion of West Virginia's control program seems to have been acceptably effective.

TREATING OAK WILT TREES TO PREVENT OVERLAND SPREAD

This section deals primarily with the prevention of disease spread because the cure of individual diseased oaks affected by *Ceratocystis fagacearum,* though conceivable (Hoffman, 1951), has not yet been made possible. Any claims made currently of an effective cure for individual oaks already diseased with oak wilt should be ignored. The research on preventive treatments designed to reduce or eliminate the chance of later natural infection in treated trees was first considered to be giving promising results. Later reports have been less encouraging (Hoffman, 1954).

The presence of fungus mats beneath the bark of many trees affected by oak wilt was reported by some early workers (Curl, Stessel, and Zuckerman, 1952; Kuntz, Parmeter, Ross, and Riker, 1952; Hepting, Toole, and Boyce, 1952 a). Research in West Virginia (Leach, True, and Dorsey, 1952; True, Staley, Leach, Barnett, and Dorsey, 1952), in Iowa (Norris, 1953, 1955; Englehard, 1955), and later in a number of other states pointed to the importance of the fungus mats, exposed through the cracking of the bark of dying trees by fungus pressure cushions, as the chief source of inoculum for overland spread by insects. In states where oak wilt mats are frequently produced, the first objective in control procedures has been to destroy the diseased trees before these mats could form, to prevent mat formation on diseased trees (Morris, 1955; True and Gillespie, 1956), or in some cases (Boyce, 1954) to reduce the degree of mat infestation by insects through the application of an insecticide to the bark of felled trees.

In Missouri, fungus mats are not often found (Buchanan, 1957), and reports from other states suggest that in some seasons, at least, oak wilt mats are produced infrequently. Yet overland spread occurs in these states. If this means that there are other unrecognized sources of overland spread, mat-inhabiting insects may not be the sole vectors of oak wilt under these conditions, and control aimed solely at the prevention of mat production can hardly be considered adequate. In Ohio (Redett, 1959) and in Arkansas (Wilson, 1959), experimental control has included one or more procedures designed to prevent possible spread of the disease by twig-feeding bark beetles. Such treatments may include the normal utilization of commercially valuable parts of the trees and

the piling and burning or spraying of the twigs and smaller branches. Preliminary studies in Ohio suggest that this system may bring about reduction in overland spread. It seems likely that in areas where fungus mats are known to form regularly, these natural reservoirs constitute the chief, but possibly not the only, source of inoculum for overland disease spread.

Control programs aiming specifically at the elimination or reduction of mat formation may also achieve some control of possible alternative means of disease transmission. This may be brought about by shortening the time during which insects can multiply in the dying wood, and by limiting the longevity of *Ceratocystis fagacearum* in twigs and branches, as well as in the trunk of treated trees. There is, however, little supporting evidence of this as yet (Merek and Fergus, 1954; Shigo, 1958).

In West Virginia, as in several other states, the first treatment designed to check overland spread from known oak wilt centers was to cut and burn the affected trees (True, Craig, and Cuppett, 1955); the trunk and branches were often piled around the diseased stump to burn them. This proved to be costly and time consuming. Moreover, it often resulted in injury to nearby trees as the result either of felling or burning the diseased tree. Fresh wounds created in this way could serve as infection courts if entered by any infested vector insects which escaped the burning or were present in the vicinity. In practice, the logs were not always completely burned or charred, and where parts of partially burned logs were left with some bark unburned, fungus mats sometimes developed. Burning diseased trees in the forest at certain seasons also was unsafe because of the forest fire danger involved.

The time-consuming, and in many ways undesirable procedure of cutting and burning, was not long continued. Studies of alternative methods for the control of overland spread were undertaken in Pennsylvania, West Virginia and at the Southeastern Forest Experiment Station in Asheville, North Carolina. Although research workers in Pennsylvania have experimented with several control treatments, utilizing fungicidal and silvicidal chemicals, the control agency early adopted, and has maintained, the practice of felling oak wilt trees and allowing them to dry out on the ground (Morris, 1955). To speed the drying process, the felled trees were frequently allowed to lie undisturbed as they fell, so that the trunk would not usually lie flat on the ground, but would be supported at least at one end by the branches. The butts of small trees were raised and placed on the stump where this was practical. These precautions, and the practice of leaving the leafy branches attached to the tree, were designed to favor rapid and uniform drying.

Trees felled late in the season were often peeled to give further assurance that fungus mats would not form before the trunk and larger branches were too dry to produce them. Federal workers at Asheville also cut the oak wilt tree (Boyce, 1954 c), and, in addition, sprayed the trunk and the larger branches with an insecticide-fungicide spray (Boyce, 1954 b). The wood was sometimes bucked and racked up before spraying. The spray was also applied to the piled brush. These sprays did not prevent mats from forming, but when mats were present, and exposed by the cracking of the bark, the sprays reduced the insect population on exposed mats to approximately an eighth of that found on mats produced on unsprayed trees. In North Carolina and Pennsylvania it was found that wet weather sometimes allowed mats to be formed in considerable numbers on the felled trees (Boyce, 1957).

In West Virginia and Pennsylvania, studies were made of the effect of chemical treatments on diseased trees (Morris, 1955; Fergus, Cole, and Stambaugh, 1955; True, Craig, and Cuppett; True, Judy, and Ross, 1955). After preliminary tests with several chemicals, a comparison was made in West Virginia between the effect upon fungus-mat formation of simply girdling the diseased trees to the heartwood and of injecting a saturated aqueous solution of copper sulfate at such deep girdles, with several untreated trees left as controls. The results are shown in Table 9. It is clear that although the injection of copper sulfate had some effect in reducing mat production, the trees girdled to the heartwood with the bark peeled below the girdle (Figure 34) produced even fewer mats.

Following these encouraging results, the use of the deep girdle was tested extensively during 1955 (True and Gillespie, 1956). Thirty-seven oak wilt trees which had been deep-girdled in June and July were felled and examined in late August. Fifty additional diseased trees girdled in August and early September were felled and examined during October and early November of the same year. Early-season girdling favored rapid drying, and small trees dried out more rapidly after girdling than larger trees. Three of the 36 trees (8 per cent) girdled during June and July, and found sufficiently dried out to examine in August, had produced one or more mats when examined. Nine of the 41 trees (22 per cent) girdled during August and September, and found dry enough to examine when felled in October and November, had produced mats at that time.

This represents a considerable reduction below 50 per cent of untreated trees which usually produce mats in this region. This is an estimate only, because no quantitative figure based on an adequate

Table 9. Abundance of Fungus Mats as Related to Treatment and Tree Size

Treatment	Tree Number	Species of oak	Diameter at breast height (inches)	Fungus mats found			
				Many	Several	Few	None
None*	3218	Scarlet	22	X			
	3361	Red	13			X	
	3325	Black	12	X			
	3423-A	Red	8			X	
	3423-B	Red	6			X	
	3140	Chestnut	3				X
			Av. 11				
Copper sulfate:	3137	Black	22		X		
saturated solution in deep	3155	Black	20				X
basal girdle at rate of	3168	Scarlet	16		X		
0.4 gallon per diameter	3159	Scarlet	16			X	
inch.	3220	Black	12		X		
	3157	Red	8			X	
	3139	Red	8				X
	3052		Av. 15				
Deep girdle to heartwood:	3219	Black	36			X	
stump peeled below girdle	3217	Red	32				X
	3214	Scarlet	30				X
	3136	Red	15				X
	3141	Red	6				X
		Red	4				X
			Av. 21				

*Includes only those untreated checks whose bark and wood were dead enough to allow fungus mats and bark cracks to form. The bark and wood of half of the untreated controls were still too green to allow mats to form. This was not true of any of the treated trees in this experiment.

sample has yet been obtained, due to the zealous industry and enthusiasm of the oak wilt control agencies at work in the Appalachian states.

The summer season of 1955 was comparatively dry, and it is of considerable interest to note that in comparable studies made following the wet summer of 1956, the figures reported (Gillespie, Shigo, and True, 1957) closely approximated those obtained in 1955. Five of 48 trees (10 per cent) deep girdled during June and July and 15 of 75 trees (20 per cent) deep girdled in August were found producing mats when examined in 1956. The comparative constancy of the corresponding percentages during one dry and one wet season seems encouraging, especially when contrasted with the marked increase in mat formation reported on felled oak wilt trees following a wet summer season (Boyce, 1957). From the West Virginia studies reported above, it was also possible to show that the reduction in mat formation was especially marked in the case of diseased trees girdled soon after symptoms had

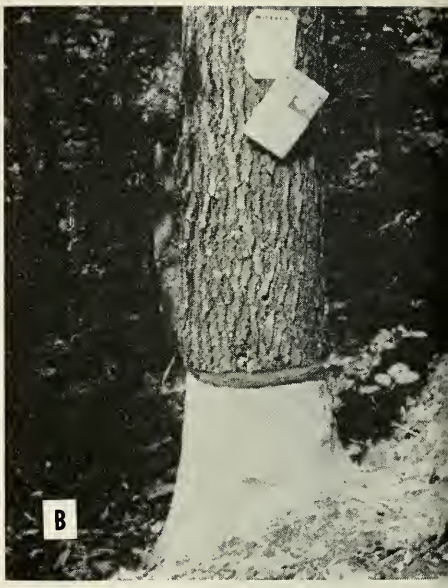

FIGURE 34. A. An oak-wilt tree girdled to the heartwood with the bark removed below the girdle. This is standard control procedure for preventing the spread of oak wilt in West Virginia. Instructions are nailed to every deep-girdled tree (B), asking the land owner not to cut the tree before December 1. A brief illustrated folder describing the disease is also attached to the tree.

appeared, as measured by percentage defoliation recorded at the time of girdling. There was at least a suggestion that black oak produced oak wilt mats less frequently after girdling than did either red or scarlet oak.

Although the deep girdle treatment has not completely prevented mat formation in treated trees, the ease of its utilization has made it an effective tool, especially in mountainous areas. Here in particular, it is advantageous to use a treatment that can be applied at the time of field diagnosis, so that a second visit to the site to treat the tree is unnecessary. With limited finances and the manpower available it was still possible, by this means, to locate and treat most trees early enough during the summer to prevent or greatly reduce mat formation. Girdling has some advantage over treatments that require the tree to be felled because diseased trees, in falling, often wound others nearby and such wounds, especially if made in early summer, may serve as infection courts for oak wilt (Cole and Fergus, 1956; Guyton, 1952; Kuntz and Drake, 1957; Englehard, 1956; Jeffrey, 1953; Craighead, Morris, and Nelson, 1953). In the light of our present knowledge, deep girdling, especially when carried out during June or July, seems likely to give the most effective

control of overland spread per dollar spent. The reasons for the effectiveness of deep girdling are not clearly understood, but research relating thereto has made good headway recently, as will be reported later in this bulletin.

OAK WILT CONTROL MEASURES DESIGNED TO CHECK ROOT-GRAFT SPREAD

It is interesting to note that when oak wilt was found in West Virginia in 1951 the only available control measures that were based on research results were those designed to prevent local spread of the disease through root grafts. Pioneer work in Wisconsin and in Iowa had suggested that this mode of disease spread might be stopped through the mechanical isolation of the roots of diseased trees, and sometimes those of their closest neighbors, from the root systems of healthy trees standing farther away. Thus all but the very closest healthy trees could be protected from root-graft infection by trenching around the disease areas. Tractor-drawn blades were designed for cutting roots as much as 30 inches below the soil surface, and mechanical ditch diggers were converted into special tools for oak wilt control. These ingenious procedures, suitable for use in the deep soils of the middlewest, were hardly appropriate for effective use in the more shallow and rocky soils of the Appalachian states.

More nearly suitable to our region was the suggestion that dead roots of chemically-poisoned root systems would serve as barriers to root-graft spread, and that if the root systems of all trees close to known infected trees were killed by silvicidal or, better still, fungicidal chemicals, the root-graft spread of oak wilt to healthy trees beyond this poison barrier would be prevented (Kuntz and Riker, 1950). Exploration of the relative values of a wide variety of silvicidal chemicals was undertaken at all research stations investigating oak wilt. The reputation of Ammate as a relatively harmless and moderately effective silvicide known to discourage the sprouting of stumps led not only to its testing in research studies, but also to its adoption by many states, in advance of testing, as an essential element in that part of the control program specifically aimed at the prevention of root-graft spread. Early favorable reports of the effectiveness of sodium arsenite led to its use in areas where the hazard to man, livestock, and wild game was not considered too great (Anonymous, 1952; Morris, 1955). The incorporation of a deer repellent further enhanced this chemical in the eyes of research workers, most of whom were unsatisfied with Ammate as a chemical for killing the entire root systems of treated trees or stumps (Fergus, Cole, and Stambaugh, 1955; True, Judy, and Ross, 1955; Jones and Bretz,

1958). In many cases Ammate killed only parts of the stumps and a few superficial roots. However, the toxicity of sodium arsenite to warm-blooded animals and to man led those in charge of control agencies in West Virginia and several other states to refuse to use this comparatively effective silvicide in routine control operations.

Organic growth-regulators which had proved promising as weed killers, 2,4-D and 2,4,5-T and others, were tested by several research agencies. In cooperation with the West Virginia Department of Agriculture, the West Virginia University Agricultural Experiment Station carried on cooperative field tests of the growth-regulators as well as with Ammate. The results were not encouraging. Both failed to kill the root systems sufficiently quickly or sufficiently completely and frequently permitted sprouting. In some instances new oak wilt trees were found at the edge of the so-called barrier one or two years after treatments were applied.

Certain newer organic fungicides had been shown by several workers to be extremely toxic to *Ceratocystis fagacearum* and other fungi in laboratory tests (Fergus, Cole, and Stambaugh, 1955). It was hoped that the presence of these fungicides in the wood of the roots and stems of oaks would kill the oak wilt fungus in treated diseased trees. In West Virginia a study was undertaken to test the effectiveness of one of these, Thioleutin,[15] in comparison with mercuric chloride (an extremely toxic inorganic fungicide) and sodium arsenite. Twenty inoculated trees received each treatment at the time when symptoms first appeared. All trees were injected through a completely encircling deep girdle. Every tree was allowed to take up between 50 and 100 mililiters of solution for each inch of diameter, breast height. At the end of the summer the oak wilt fungus could still be isolated from points only 1 foot above or below the injection girdle. By spring one or more stumps receiving each treatment had sprouted. It was clear that the toxicity or concentration of Thioleutin, mercuric chloride, and sodium arsenite in the areas where the fungus was present did not enable them to kill out *Ceratocystis fagacearum* in treated trees. There was a suggestion that, in nature, something prevented the fungicides from making effective contact with the fungus in at least some of the infected cells.

Studies with dyes as well as poisons had shown that it was not difficult to get reasonably good distribution of these chemicals upward through the trunk and larger branches of non-diseased oaks, but that it was extremely difficult to get the chemicals down into the deeper roots or even into the undersides of some of the surface roots. This

[15]Furnished by Charles Pfizer and Company.

could explain the apparent ineffectiveness of the poison barriers experimented with in nearly all states and widely used outside of West Virginia. In an attempt to overcome this, a radically different type of injection was attempted, in which the toxic materials were introduced into the living bark tissues (the phloem). The dead outer bark was removed over an 8- to 12-inch band around the circumference of healthy oak trees at a convenient working height. A plastic encircling pouch, sealed at the upper and lower edges of the exposed living tissues, was filled with the chemical to be tested before the top was sealed (Figure 35). It was hoped that the experimental chemical would be slowly absorbed by the living tissues of the inner bark and carried downward into the living bark of even the small roots. Dilute concentrations were purposely used so that the chemicals could become widely distributed within the tree before exerting their lethal effect. The chemicals tested in this way included a water suspension of chlorinated methyl urea (CMU), the chelated organic copper compound, cupric-ethylenediaminetetracetic acid, and trichlorobenzoic acid (TBA), as well as 2,4-D and 2,4,5-T. Both sugar and boron were used as additives in some cases, since such

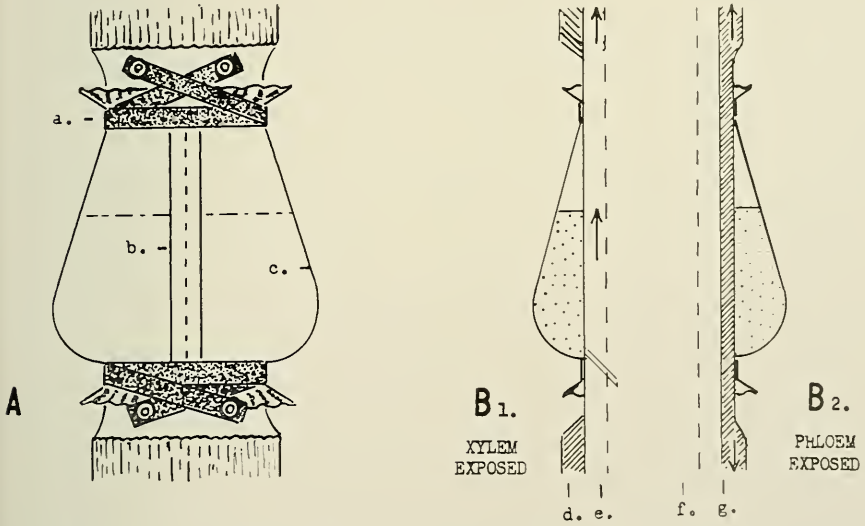

FIGURE 35. An encircling plastic pouch for the introduction of solutions or suspensions into the trunks of trees. A. External view showing the trunk with the bark shaved away to the desired depth and the three principal components of the pouch: (a) rubber pressure bands nailed at ends, (b) the waterproof tape seal, and (c) the completed cylindrical plastic sheath. B. Cross-sectional view showing the two types of application; B_1. for xylem uptake and B_2. for phloem uptake. Arrows indicate predicted direction of transport. Letters at the bottom designate the relative positions of the bark including the conductive phloem (d), the sapwood (e), the heartwood (f) and the cambium (g).

additions had been reported to increase the effective distance to which such chemicals were sometimes carried (Mitchell and Brown, 1946; Mitchell, Dugger, and Gauch, 1953). Although the silvicides used were usually slowly absorbed by the exposed living bark, and induced symptoms in the above-ground parts of the tree, there was no evidence that any of these substances were acceptably effective as used. These studies were another example of the difficulties experienced in attempting to kill trees and their entire root systems using chemicals.

In view of the foregoing difficulties, which were rather typical of the results obtained by most oak wilt research workers seeking a chemical control of oak wilt spread, the question of the need for, or desirability of, attempting to control root-graft spread in West Virginia and in other areas of the Appalachian region was re-examined. Early preliminary surveys had shown that in the areas of West Virginia where sample plots were established around oak wilt sites, there were, within fifty feet of the isolated, diseased tree, approximately eight healthy oaks averaging 10 inches d.b.h. and belonging to species whose roots could be expected to graft with those of the diseased tree. If all chances of root-graft transmission were to be eliminated, eight such healthy oaks would have to be killed for each new case of oak wilt resulting from overland spread. This could well mean that in attempting to prevent root-graft spread at each oak wilt site, the control efforts would be killing a good many more trees than the disease. Even so, if such treatment could be counted on to prevent further disease spread at the site, so that the infection area need not be revisited in succeeding years, it might be worth doing, especially in inaccessible areas where periodic revisitation is difficult.

However, the effectiveness of treatments designed to check root-graft spread has been doubtful not only in West Virginia, but elsewhere. In Missouri (Jones and Bretz, 1958), local disease spread in experimental plots was greater after treatment of nearby healthy trees with Ammate and with 2,4,5-T to establish a poison barrier than in plots where no attempt had been made to check root-graft spread. This could have resulted in part from failure of the treatments to kill the entire root systems of the healthy trees within the barrier. Death of the known diseased tree, either from disease or from treatments, might be expected to reduce its rate of water loss so that the current of the sap stream through existing root grafts would be increased in the direction of the healthy trees grafted to it. *Ceratocystis fagacearum* could perhaps grow saprophytically through the killed roots of poisoned trees to the grafted root systems of untreated healthy trees beyond. Crossing such a barrier might slow up the spread of oak wilt without stopping it. Probably

either imperfect root kill or saprophytic growth through poisoned root systems could be responsible for the failure of the chemical barrier zone to prevent the local spread of oak wilt through root grafts. The possibility should be borne in mind that considerable local intensification may result from short-distance overland spread by insects. Insect population may build up locally in diseased trees or in cut or poisoned trees close by in cases where a "poison barrier" is used.

In the meantime long-term observations of the results of mechanical severance of roots has shown that regrowth and regrafting of severed roots seem to make this proposed control for root-graft spread only temporarily effective.

Studies in West Virginia on the translocation of dyes and poisons demonstrated the presence of root grafts between an injected oak and closely related oaks around it in approximately 10 per cent of the 75 oaks tested. The greatest distance between trees found united by root grafts was 29 feet, and usually trees found root grafted were only a few feet apart. It seems likely that the technique used would serve to indicate the presence of most, but not all of the root grafts, chiefly those through which rather large volumes of the experimental chemicals had passed from the treated tree or stump to the untreated root system united with it. Perhaps root grafts of some sort may unite as many as 20 per cent of our diseased oaks with their healthy neighbors. Root-graft spread will not always occur even where root grafts are present. Although Wisconsin workers have shown that the oak wilt fungus, like dyes and poisons, is capable of spreading through the grafts uniting grafted systems (Kuntz and Riker, 1950), other investigators have not yet obtained comparable results. It may well be that root-graft spread actually occurs only occasionally.

The reasons why West Virginia has never adopted, as part of its control program, any treatment designed specifically to stop root-graft spread are: (1) The mechanical severence techniques are not usually applicable under forest conditions in West Virginia; recently these have been shown to be only temporarily effective; (2) The attempted establishment of poison barriers did not appear to succeed in our own experimental studies. Most other investigators except Kuntz and Riker (1950) have shared this experience; (3) The effectiveness of poison barriers as used in control programs in other states appears questionable; (4) The need for and the advantage to be gained from undertaking expensive measures aimed specifically at the control of root-graft spread seem not to be great enough to justify the cost involved (this is especially true because surveys designed to pick up additional cases of overland spread will

incidentally pick up trees which become diseased through root grafts); and (5) Many healthy trees near known oak wilt trees would be sacrificed in such an attempt.

The decision not to attempt to control root-graft spread in West Virginia for the present means that revisitation of known oak wilt sites is an important part of the oak wilt control program here. A small and decreasing percentage of the known infection centers in West Virginia have shown new disease during the first, second, and third years after treatment. After five years of continuous freedom from disease, sites almost never become reactivated. This means that after five years of observed absence of the disease, these infection areas need not be revisited. It has been found, however, that some few infection centers are characterized by continuing disease recurrence at short intervals. Infection centers which are multiple-tree infection centers at the time of discovery are especially likely to show repeated disease recurrence. The existence of these centers emphasizes our continuing need for a really effective method by which the local spread of oak wilt may be stopped. The use of such a method might be reserved for multiple-tree sites or for those particular sites at which the disease recurs repeatedly. Possibly a more effective method of checking long-distance spread could also be expected to reduce further the incidence of local spread.

HOW THE DEEP-GIRDLE TREATMENT CHECKS THE FORMATION OF OAK WILT MATS

The effectiveness of the deep-girdle method in preventing mat production has been established and measured. We know that it is not completely effective as a way of preventing oak wilt mat formation. We believe that it shortens the survival time of *Ceratocystis fagacearum* in dying trees. We did not know why deep girdling had this effect. The first assumption was that the deep-girdle treatment caused trees to dry out so fast and so completely that oak wilt mats in most cases had insufficient time to form. No one will question that one result of deep girdling is to reduce the moisture content of the stem above the girdle. Campbell and French (1955) have shown that the moisture content of non-girdled diseased trees does not decrease markedly after symptoms appear and, in the case of trees which form mats the year symptoms are shown, is still high just before the mats are produced. Probably this is due partly to a reduction in the rate of water-loss from the crown as the leaves die and fall to the ground. The roots, whose normal functioning it not at first affected, probably continue to absorb water after the branches lose their leaves. For a time this water collects in the wood

of both roots and stems since there is no place where it can be effectively lost through evaporation. It is only after the pressure cushions crack the bark of non-girdled trees which produce mats the same year in which they show symptoms that the water content of the trunk is reduced. It is clear that early drying out after deep girdling would force mats to form soon after treatment, if they are to form at all, especially in diseased trees girdled during the early summer.

Limited studies of the relationship of moisture content to treatment showed that water content was higher for non-girdled trees. Trees injected with copper sulfate through deep girdles and trees receiving only the deep girdle produced fewer mats and had a lower water content (Table 10).

Trees injected with copper sulfate showed little or no sapwood decay when compared with those which received only the deep-girdle treatment.

The greenish discoloration associated with the copper sulfate treatment seemed to be darker and to ascend higher in the deeper annual rings than in the superficial rings where *Ceratocystis fagacearum* had established itself before treatment.

These observations suggested that reduced moisture content of deep-girdled trees was not the sole factor limiting mat formation, but that the entrance of competing fungi into the girdled trees which received no chemical treatment supplemented the effects of the lowered

TABLE 10. RELATIONSHIP OF PERCENTAGE MOISTURE (DRY-WEIGHT BASIS) OF SAPWOOD TO TREATMENT AND TO SEASON OF HARVEST FOR OAK WILT TREES

TREATMENT	SEASON OF HARVEST*	NUMERICAL BASIS		PERCENTAGE MOISTURE OF THE SAPWOOD	
		BLOCKS**	TREES†	AVERAGE	RANGE
Non-treated Controls	Early	4	2	91	72-104
	Late	3	3	72	64-81
Deep girdle	Early	4	2	79	61-98
	Late	2	2	57	40-74
Copper Sulfate	Early	6	3	74	60-86
	Late	4	3	71	55-82

*Early harvest designates felling and sampling in late August and early September. Late harvest designates an October date.

**Sample blocks 1 ft. long were sawed from the trunks of felled trees at points 3 ft. and 6 ft. above the girdle of treated trees and at corresponding heights in nontreated controls. Since moisture content seemed not to differ at these heights, samples taken at both heights are included here. Four sub-samples were taken from each block for moisture determination.

†Trees sampled all belonged to the red-oak group. Half of them were red oaks, and scarlet and black oaks made up the rest of the sample.

moisture content in reducing the rate of mat production. The presence of the injected copper sulfate presumably reduced the attack of the competing fungi in chemically-treated trees. Copper sulfate seemed unable to penetrate the vessels of the outer rings sufficiently to kill the oak wilt fungus there. The tyloses and gums, induced by the presence of *C. fagacearum,* presumably interfered with the penetration of the chemical but appeared to be no barrier to competing fungi, some of which had already caused sapwood decay in the outer rings of trees girdled without chemical treatment (Figure 36).

The interaction of *Ceratocystis fagacearum* with a number of other fungi isolated from diseased oaks was looked into by researchers in other states (Brandt, 1953; Young and Brandt, 1953; Turk, 1955), but little of practical or fundamentally significant value developed. Shigo (1958) undertook in 1956 and 1957 to determine what fungi were associated with *Ceratocystis fagacearum* at various stages of disease development and for a short period after the death of oak wilt trees in West Virginia. With the help of State Department of Agriculture personnel he was able to obtain and study approximately 5,000 Petri-dish cultures made from 1,158 samples taken from more than 400 oak wilt trees, most of which had received the deep-girdle treatment in routine control operations. Of more than 50 fungi isolated, nine were found with considerable regularity. These received intensive laboratory study. Each was paired with *C. fagacearum* in laboratory culture to determine the interaction. Four had little or no effect upon the oak wilt fungus when grown with it on conventional media. Five other fungi, including *Gliocladium roseum* Bain., *Hypoxylon atropunctatum* (Schw) Cke., *Trichothecium roseum* (Pers.) Link, *Penicillium* sp. and *Trichoderma lignorum* (Tode) Harz., overgrew *C. fagacearum,* either killing it or checking its growth. *G. roseum,* which seemed to have the most consistently destructive effect on contact or in close association with *Ceratocystis fagacearum* and other fungi with which it was paired in culture, was chosen for field testing. Experimental trees were injected or inoculated with spores of *G. roseum* as soon as the first symptoms of oak wilt appeared. As used in the field, *G. roseum* failed to spread extensively within the treated trees and thus did not exert any demonstrable control of the oak wilt fungus. Shigo observed, however, that *Hypoxylon punctulatum* (Berk. and Rav.) Cke. or its conidial stage (Barnett, 1957) infected the girdled trees and became extensively established in them. A white rot of the sapwood followed infection, and ultimately the fruiting bodies of *Hypoxylon,* or its imperfect stage (*Basidiobotrys* sp.), were produced on nearly all deep-girdled trees.

FIGURE 36. A. The fruiting bodies of different species of the fungus genus *Hypoxylon* often appear on the trunks of deep-girdled oak wilt trees. B. When *Hypoxylon* infects the wood above the deep girdle it induces a fast-spreading white rot with zone lines. It is seldom possible to isolate the oak wilt fungus from wood in which *Hypoxylon* has become established.

Ceratocystis fagacearum could seldom be isolated from wood in which *Hypoxylon punctulatum* had become established.

Although *Hypoxylon punctulatum* was one of the four common fungi that Shigo had found to cause no unfavorable effects upon *C. fagacearum* in laboratory studies using paired cultures on conventional media, he suggested the need for further study of this fungus, which occurred so frequently and seemed to exert some limiting action upon the oak wilt fungus in nature.

Roncadori (1958) confirmed and extended Shigo's observation by showing that on conventional media neither *Hypoxylon punctulatum* nor sapwood rotted by it produced any inhibitory antibiotic effect on *Ceratocystis fagacearum* grown in the same Petri dish (Figure 37). When he added cellulose or the finely-ground bark or sapwood of healthy oaks to artificial media in which *C. fagacearum* and *H. punctulatum* were grown together, the subsequent growth of the *Hypoxylon* was much greater than that of the oak wilt fungus, which cannot utilize

FIGURE 37. When a block of wood decayed by *Hypoxylon punctulatum* was placed in a Petri dish between growing cultures of the oak wilt fungus, *C. fagacearum* grew up to and over the surface of the block. Neither *H. punctulatum* nor wood decayed by it seemed to exert any repellent effect upon the oak wilt fungus (Roncadori, 1959).

cellulose for growth. *C. fagacearum* was unable to make more than sparse growth on an agar medium composed of sapwood rotted by *H. punctulatum,* but made excellent growth if favorable carbon and nitrogen sources were incorporated (Figure 38).

It is evident that the rapidly growing *Hypoxylon punctulatum,* which can utilize cellulose as a carbon source, quickly exhausts also the carbon and nitrogen sources required by *Ceratocystis fagacearum,* resulting in actual starvation of the oak wilt fungus. Although *H. punctulatum* seems to produce no toxic substance antibiotic to *C. fagacearum,* it has been shown to hold a tremendous nutritional advantage over the oak wilt fungus under laboratory conditions corresponding closely with conditions which exist in nature when both fungi are present together in girdled oaks.

Field tests made in 1958 showed that *Hypoxylon punctulatum* enters most deep-girdled trees at the girdle wound, that it spreads rapidly upward through the trunk, and that it may enter the larger branches (Roncadori, 1959). Presumably it can enter through other wounds extending through the bark since it has been found frequently in non-girdled trees. Of several application methods tested, the most effective appeared to be a spray application of suspensions of spores or of spores and mycelium to freshly-made girdle wounds. Although *H. punctulatum* also entered most of the unprotected non-inoculated trees, apparently through the girdle, the *Hypoxylon* was more uniformly and extensively distributed within the tree when it was sprayed on the girdle

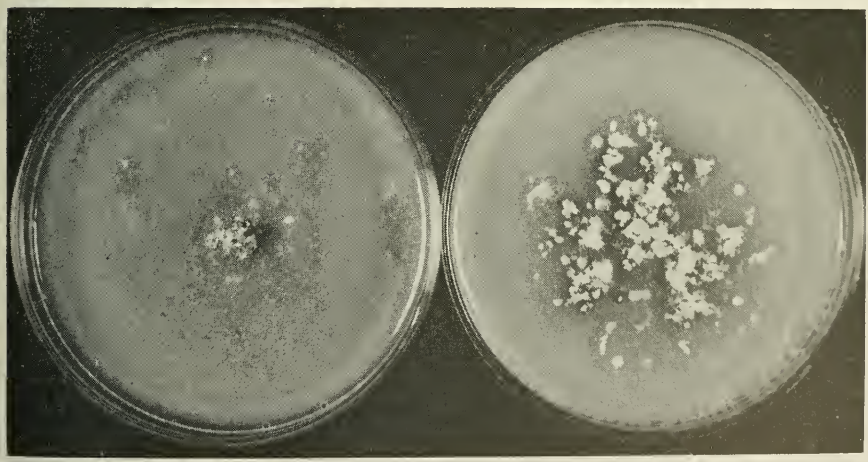

FIGURE 38. When the oak wilt fungus was grown on a medium consisting of pulverized rotted wood it made very little growth (left), but when suitable nutrients were added, the good growth shown in the Petri dish on the right was induced. (Roncadori, 1959).

wound. Conversely, the oak wilt fungus was more consistently limited to the branches of trees given *Hypoxylon*-treated girdle wounds than in deep-girdled trees not sprayed with *H. punctulatum*. Field studies are planned on a larger scale to test further the value of applying inoculum of *Hypoxylon punctulatum* to deep-girdled oak wilt trees.

Although *Hypoxylon punctulatum* has never been reported to be parasitic, other members of the genus have been reported capable of causing die back in oak and other species, particularly in drought years. With this in mind, large and small healthy oaks belonging to the red- and white-oak groups were wounded and inoculated with spore suspensions or spores and mycelium of *Hypoxylon punctulatum, Hypoxylon atropunctatum* and *Hypoxylon mediterraneum* de Not., all of which occur naturally on oak in West Virginia. None of the trees showed symptoms at any time during the summer season (1958). When the trees were sampled and cultured in the fall, *H. punctulatum* was not reisolated. One sample each, taken from trees inoculated with *H. atropunctatum* and with *H. mediterraneum,* yielded these fungi on culturing. Tests of the pathogenicity of *Hypoxylon* species to healthy oaks will continue since it is conceivable that even *H. punctulatum* might prove to be weakly parasitic in drought years or on weakened trees.

ENLISTING ECOLOGICAL FACTORS TO HELP CONTROL OAK WILT

We know that *Ceratocystis fagacearum* is a virulent and destructive parasite while it lives in the vessels of healthy oaks of the red-oak group and causes these trees to show the symptoms of oak wilt and to die. Barricaded within these vessels by the tyloses and wound gums, which its presence induces, the fungus is most difficult to kill with fungicidal chemicals. We know that under some conditions the oak wilt disease is capable of spreading for long, though undertermined, distances over land, and of spreading locally to cause disastrous losses by its intensification at established disease centers.

And yet we begin to understand that the oak wilt fungus leads a precarious existence. There are critical times when even slight modifications in its environment may lead to its success or failure, may make possible or prevent its spread. We need to know more about how to take advantage of these crisis situations in controlling the disease.

Although *C. fagacearum* lives within the wood vessels of infected trees, it cannot utilize cellulose (Roncadori, 1959). Although it is reported to produce an enzyme capable of dissolving the pectic compounds of the cell wall, Kessler (1959) has reported that it makes little or no nutritional use of these cell wall substances. While the tree is still

vigorously alive, the food supply of the oak wilt fungus seems to be limited to extremely low concentrations of sucrose and a few amino acids presumed to be present in the vessel sap (Roncadori, 1959). The period of its life which follows immediately after infection could well be critical for its survival, as suggested by the frequent survival of only one fungus type in trees inoculated with two or more distinct types (Barnett and Jewell, 1954). Recent studies (Struckmeyer, Kuntz, and Riker, 1958) have indicated that as the disease progresses, the fungus is able to penetrate the living parenchyma cells of the rays where higher concentrations of a wider variety of food substances may be available. In the late stages of disease, as the parenchyma cells of bark and wood begin to die, there seems to be a two-way radial migration of the mycelium, with massive mycelial development in the inner stem bark where food is abundant. Now, for the first time (and only briefly), the oak wilt fungus has available to it and to it alone, the tremendous nutritional resources liberated by the dying cells of the affected trees. The development of pressure cushions and of mycelial mats between the bark and wood may follow; often it does not. We still do not know the factors which activate, restrict, or prevent their development. If cushions and mats are not produced, one important way by which the disease may spread has failed to materialize.

The deep-girdle treatment appears to suppress mat formation in at least two ways: by limiting the water supply, and by permitting the entrance and spread of competing fungi. It seems that although infection by *Hypoxylon punctulatum* may require or be favored by moist conditions, the extensive spread of the fungus within the tree, and the development of the associated rot are favored by the drying out of the girdled tree. The oak wilt fungus situated in girdled trees faces, then, a very critical situation when the parenchyma cells of the host die, liberating an abundance of foods suitable not only for its use but also for the use of the less fastidious invading saprophytic fungi. Perhaps those secondary fungi frequently use so much of the available food, needed in abundance by *C. fagacearum* to produce the pressure cushions and fungus mats, that their production is limited and the spread of oak wilt by this means is often prevented. By girdling oak wilt trees, we tip the natural balance in this struggle against the oak wilt fungus, and enlist the help of competing fungi such as *Hypoxylon punctulatum* in our control program.

This may not be all we have done by deep-girdling oak wilt trees. Many deep-girdled trees become naturally infected with species of *Hypoxylon* and these fungi often fruit abundantly on them. The fruiting bodies of *Hypoxylon* are produced in stromata which form at the

cork cambium region between the outer bark and the phloem tissues in much the same way that the pressure cushions and mycelial mats of *C. fagacearum* form at the vascular cambium, between bark and wood. Many insects which inhabit oak wilt mats are also found on the stromata of *Hypoxylon punctulatum,* so that presumably the spores of each fungus may be carried to the fruiting structures of the other, and both may be introduced, separately or together, into the fresh, sappy wounds that attract certain of these insects to freshly injured, healthy trees. The moist environment of the fresh wound would seem to favor the germination of the spores of both fungi. Conditions within the moist sapwood could easily favor the initial extensive spread of the oak wilt fungus, but if *Hypoxylon* should infect and survive until the wood begins to dry out from the effects of the oak wilt disease, the *Hypoxylon* may subsequently spread extensively through the tree to compete with and limit the saprophytic development of *C. fagacearum.* Perhaps other fungi also may be similarly introduced by vector insects at the time when they infect the trees with oak wilt (Shigo, 1958) or by other insects visiting oak wilt mats. The saprophytic development of such fungi could be a natural factor which might exert some limiting effect on the production of fungus mats in some sections of the country, even in non-girdled oak wilt trees (Wood and Peterson, 1959). Competing fungi, introduced on fungus mats by visiting insects, may shorten their period of sporulation (Figure 39).

It seems reasonable to assume that the presence of a large quantity of *Hypoxylon* inoculum in the near vicinity of newly diseased and freshly girdled oak wilt trees will favor their early natural infection by *Hypoxylon.* It is possible that the conidia of *Hypoxylon* may be effectively wind-borne so that the insects present on the sporulating stromata need not be the sole means by which such spores may be carried to the deep-girdled wounds or to other suitable infection courts.

There are yet other ways in which the use of the deep girdle may assist in the control of oak wilt by favoring environmental factors detrimental to *Ceratocystis fagacearum.* Temperatures within the wood of trees deep girdled in summer may be expected, after girdling, to follow more closely the temperature fluctuations of the surrounding air. At times during the summer, temperatures within the wood of girdled trees may approach the critical upper limit at which *C. fagacearum* will grow, or perhaps even survive (Bretz and Morison, 1953; Merek and Fergus, 1954). The same would be true in tissues on the exposed upper sides of felled trees, but on their lower shaded sides the temperature would, in summer, presumably be constantly lower than on any side of standing

FIGURE 39. Sometimes the fungus *Trichothecium roseum* develops on the fungus mats of the oak wilt fungus and fruits in the bark cracks and elsewhere on the bark in the vicinity of the mats. *Trichothecium roseum* is antagonistic to the oak wilt fungus when grown with it in the laboratory and it may exert a similarly antagonistic effect in nature.

deep-girdled trees. It is interesting to note that *Hypoxylon punctulatum* will grow at higher temperatures than *C. fagacearum*.

Standing deep-girdled trees would be expected to dry out as rapidly and more uniformly than felled trees. This could be reflected in differences in mat production following rainy summers. Although mats do occasionally form on oak wilt trees felled during dry seasons, they have been reported to form much more frequently on such trees felled during seasons that are wet (Boyce, 1957). In the case of deep-girdled standing trees, the evidence of two years indicates little fluctuation in mat production following a wet and a dry summer (True and Gillespie, 1956; Gillespie, Shigo, and True, 1957).

It may be pointed out that the ecological approach to the control of tree diseases has received little attention. This is in part due to the fact that such diseases as chestnut blight, phloem necrosis of elm and, perhaps the Dutch elm disease, spread so rapidly and so aggressively that inhibitory ecological influences could hardly be enlisted in time to be effective. Recent studies (Van Arsdel, Riker, and Patton, 1956) on the effects of temperature and humidity upon the germination of the spores of *Cronartium ribicola* indicate that under certain specific and limited climatic conditions the white pine blister rust cannot complete its life cycle even in the presence of the alternate host (*Ribes* spp.). Where this condition exists, the eradication of currants and gooseberries to control of the blister rust on pine is unnecessary. The study of ecological factors has here pointed to a possible reduction in the over-all cost of control. Ecological studies of *Ribes* spp. in the West have made it possible for silvicultural controls to supplement *Ribes* eradication (Davis and Moss, 1940).

The deep-girdle method for suppressing oak wilt by checking its spread overland is the cheapest of any currently used on a large scale. Its simplicity and ease of prompt use enhance its effectiveness. It promises to be as effective as any that may be safely used in a wide variety of cultural situations.

It is clear, however, that even in coming this far along the path toward the utilization of ecological factors to help suppress oak wilt, the forest pathologists have required and received essential technical help from men working in the related fields of mycology and entomology. Microclimatic studies on standing and felled trees are under way. Physiological and genetic studies of *C. fagacearum* and of competing fungi within diseased trees should play important parts in future studies related to oak wilt control.

Clearly, then, the utilization of potentially favorable environmental factors will require continued research in forest pathology, forest entomology and in related fields. There are things to be guarded against

as well as advantages to be taken. In neither case can we make wise choices if we are uninformed concerning the basic factors involved. The results of our control procedures are not likely to be uniform over wide areas because of differences in the various environments themselves. The ultimate results will not be clear immediately, and the need of financial support for research and control will continue.

It is doubtful whether complex expensive programs of control can be long continued unless they are regarded as essentially temporary measures. In the case of oak wilt we must look forward to an indefinitely long period of continued survey and disease control. The cheapest method that will gain an acceptable degree of suppression over the years seems now to be one which will enlist favorable forces of the environment on our side. It is our job as research scientists to investigate these forces, and to determine which of them can be trusted to act consistently for us.

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