

# **PROCEEDINGS OF THE 26th ANNUAL WESTERN INTERNATIONAL FOREST DISEASE WORK CONFERENCE**

**Tucson, Arizona  
September 1978**



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PROCEEDINGS

JOINT MEETING

TWENTY-SIXTH ANNUAL WESTERN FOREST INSECT WORK CONFERENCE

TWENTY-SECOND ANNUAL WESTERN INTERNATIONAL FOREST DISEASE WORK CONFERENCE

Monterey, California

February 23-28, 1975

EXECUTIVE COMMITTEE (Twenty-Sixth WFIWC Conference)

G. C. Trostle, Boise	Chairman
R. E. Stevens, Fort Collins	Immediate Past Chairman
G. D. Amman, Ogden	Secretary-Treasurer
W. G. H. Ives, Edmonton	Councilor (1972)
R. G. Cox, Lewiston	Councilor (1973)
L. Safranyik, Victoria	Councilor (1974)

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K. M. Swain, San Francisco	Program Chairman
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EXECUTIVE COMMITTEE ELECT

G. C. Trostle, Boise	Chairman
R. E. Stevens, Fort Collins	Immediate Past Chairman
G. D. Amman, Ogden	Secretary-Treasurer
R. G. Cox, Lewiston	Councilor (1973)
L. Safranyik, Victoria	Councilor (1974)
D. L. Parker, Albuquerque	Councilor (1975)

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B. E. Wickman, Corvallis	Program Chairman
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## FOREWORD

The Twenty-sixth Annual Western International Forest Disease Work Conference met at the Arizona Inn in Tucson on October 24-28, 1978. Since the meeting was scheduled a week prior to the annual meeting of the American Phytopathological Society, also held in Tucson, we were fortunate to have several visitors from the East.

The Arizona Inn provided a unique atmosphere for the meeting, which was called to order by Chairman Dick Smith on Tuesday morning. A welcome was extended to us by Darrel S. Metcalfe, Dean of the College of Agriculture at the University of Arizona, and the keynote speech was delivered by Erwin H. Zube, Director of the School of Natural Resources at the University of Arizona.

Mrs. Gilbertson organized several shopping and sightseeing trips around the Tucson area for the wives. The wives greatly appreciated Mrs. Gilbertson's time and effort to make sure their visit to the Tucson area was a memorable one.

The usual WIFDWC-sponsored field trip was delayed until Saturday the 28th so that forest pathologists who did not attend WIFDWC but were planning to attend the APS meeting could accompany us. Because of the unique nature of the area surrounding Tucson, many diverse ecosystems were visited during the field trip, and many diseases occurring on vegetation of the area were observed.

The officers for the 26th Conference were:

Dick Smith - Chairman  
Dave Drummond - Secretary-Treasurer  
John Hopkins - Program Chairman  
Bob Gilbertson - Local Arrangements

We would like to express our appreciation to Noah Jacobs for designing the cover of this year's proceedings.

## PARTICIPANTS

Adams, D.  
Alosi, C.  
Anderson, B.  
Baker, F.  
Barnard, E.  
Beatty, J.  
Bega, R.  
Blakeslee, G.  
Blanchette, R.  
Burdsall, H.  
Byler, J.  
Campana, R.  
Cobb, F.  
Dooling, O.  
Dorworth, D.  
Drummond, D.  
Dunn, P.  
Filip, G.  
Florance, E.  
Foster, R.  
Fuller, L.  
Gilbertson, R.  
Gillman, L.  
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Thies, W.  
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Walla, J.  
Weir, L.  
Wicker, E.  
Williams, R.  
Wood, E.  
Worral, J.  
Zabel, R.  
Zagary, D.

## IN MEMORIAM

Wilhelm G. Solheim

Dr. Wilhelm Gerhard Solheim, Emeritus Professor of Botany at the University of Wyoming, died in Laramie, Wyoming, on May 15, 1978, after a long illness. He had reached his 80th birthday on May 13.

Dr. Solheim was born in Stoughton, Wisconsin, and had his early education there. He served in the U.S. Army during World War I as an officer in the Cavalry, stationed at Deming, New Mexico. Following his discharge from the Army he continued his education at Iowa State Teacher's College, where he earned the B.A. degree in 1924. He then entered the University of Illinois and received a M.A. degree in 1926 and a Ph.D. in 1928. His strong interest in leaf and stem parasites he retained throughout a long professional career. His Ph.D. program was directed by the legendary F.L. Stevens. One of Dr. Solheim's prized possessions was an ancient, tattered, and worn traveling salesman's sample case with several compartments, formerly the property of F.L. Stevens. Bill used this as a field plant press right up to the end of his physical ability to do field work. Dr. Solheim's main contribution to mycology was his *Mycoflora Saximontanensis Exsiccata*. This consists of 17 sets of 100 Rocky Mountain fungi issued to approximately 30 herbaria all over the world. The first set was issued in 1934 and the 17th in 1979.

Dr. Solheim began working at the University of Wyoming in 1929, and was head of the botany department from 1930 to 1950. He also served as acting dean of the College of Arts and Sciences for two years prior to his official retirement in 1963. He suffered a severe heart attack in 1951, but recovered to resume his usual vigorous field collecting and herbarium work. Although he was under constant medical care for his heart condition after that time, it had no visible effect on his enthusiastic pursuit of mycology and his lust for life in general. His retirement years were an active and exciting period. From 1963 to 1965 he directed the establishment of a college of agriculture in Afghanistan under the University of Wyoming's International Development Contract. During this period he narrowly escaped death in an automobile accident on a remote Afghanistan road. Shortly after his return in mycology and forest pathology program left vacant by the sudden death of Dr. Paul D. Kenner in mid-semester of fall 1966. Dr. Solheim taught a great deal in organizing the mycological herbarium. He was also instrumental in selecting a permanent faculty member to fill the mycology-forest pathology position. For several years afterwards he spent parts of each winter at the University of Arizona where he worked in the field, an activity he pursued with great enthusiasm and skill.

Shortly before his death, Dr. Solheim received an honorary LL.D degree from the University of Wyoming in recognition of his outstanding services to that institution.

Throughout Dr. Solheim's career his lovely and devoted wife Ragnhild was his constant companion. She will continue to reside in Laramie and is currently assisting in the organization of the Wilhelm G. Solheim Mycological Herbarium to be housed in the Department of Botany at the University of Wyoming.

There is so much that could be said about Bill Solheim but it is impossible to capture his spirit in print. He was held in high esteem by all who knew him and enriched the lives of all of us who were fortunate enough to share the pleasure of his company.

## IN MEMORIAM

Stuart R. Andrews

Dr. Stuart R. Andrews passed away on May 25, 1978, in Fort Collins, Colorado, following a lengthy illness. He was 68 years young. Stuie retired from his position as Project Leader for Forest Disease Research in the Central Rocky Mountains, with the U.S. Forest Service, Rocky Mountain Station, in November 1971. Dr. Andrews received his B.S. from Yale in 1933 and a masters in forestry from the same institution in 1935. Stuie joined the old Division of Forest Pathology in 1935 with a 30-day appointment. Stuie's aptitude for research was recognized early and his "30-day appointment" extended into a full 36-year career in forest pathology research. His early work was with Dr. L.S. Gill on red rot studies in the Black hills of South Dakota, and later on the same disease in the Southwest, stationed in Albuquerque. His career was interrupted by a 1942-46 tour of duty as a captain in the Medical Service with the U.S. Army in the South Pacific. After the war he returned to Albuquerque (following a brief assignment in Columbus, Ohio, to work on elm phloem necrosis) and continued his red rot studies which resulted in his Ph.D. dissertation at the University of Minnesota in 1953. From 1953 to 1960 he continued his red rot studies and served as Disease Project Leader in Albuquerque. His studies of red rot rates of decay, biology, and control established him not only as the authority on this, the most serious decay of ponderosa pine, but on decays in general. In September 1960, after the retirement of Lake Gill, Stuie moved to Fort Collins as Division Chief for Forest Disease Research. He served in that capacity and later as Project Leader in Fort Collins until his retirement. Although Stuie was best known for his research on red rot, he was interested in many pathological and forestry problems. For example, he also contributed to our knowledge of dwarf mistletoes, aspen diseases, pole blight, cottonwood diebacks, rusts, needle casts, Dutch Elm disease, and other diseases. Because of Stuie's broad knowledge and insight into this myriad of forest problems, he was frequently consulted and freely gave his time and advice.

Stuie was a charter member of W.I.F.D.W.C. and a very active supporter of this organization. He served as Chairman of the 1960 conference in Coeur d'Alene, Idaho, and attended as many conferences as he was able to.

Stuie was an extremely thorough and capable scientist and an excellent writer. He was noted for his good humor and ready wit, which he retained even during his last few years when he was suffering from emphysema. His tenacity in coping with his fatal disease was truly remarkable.

Stuie is survived by his wife, Helen, of Fort Collins, by a daughter, Jenné, of Minneapolis, and a son, Stuart, of Estes Park, Colorado. With Stuie's passing, the profession of forest pathology has lost a valued member. But the high calibre of his research, his professionalism, and his devotion to his family, live on as an inspiration to all of us.

## RENEWABLE NATURAL RESOURCES OF ARIZONA

Ervin H. Zube\*

### INTRODUCTION

Arizona is known as one of the sun-belt states and is a desert or arid-lands state. These are very broad and stereotypical descriptions. However, Arizona is also a state of contrasts. I would like to briefly describe the state by noting some of these contrasts before discussing the state's renewable natural resources. The contrasts are found in the physical environment, for example, climate, topography, and vegetation, and in the social or cultural environment, in the diversity of our populations.

I will also touch briefly on the issue of population growth in the state. Indeed, the rate of population growth is one of the most critical factors affecting the management of Arizona's renewable natural resources in the years ahead.

### PHYSICAL ENVIRONMENT

Ninety percent of the land area is classified as arid or semi-arid. But there are sharp contrasts within this broad physical categorization. Elevations range from 138 feet above sea level at Yuma, located in a region of desert plains in the southwest corner of the state, to a high of 12,670 feet at Humphrey's Peak in the San Francisco Mountains north of Flagstaff, near the Mogollon Rim. The Mogollon Rim, an abrupt transition slope running southeast-northwest across the entire state, separates the great Colorado Plateau of the north from a wide region of mountain ranges running generally parallel with the Rim. The Colorado Plateau and the desert plains are not without elements of relief also. The Plateau is perhaps best known for the dramatic river canyons found there (e.g., Grand Canyon) while the desert plain is marked by lower and more compact mountains than are found in the central mountain region.

Average annual rainfall varies from about 3.6 inches in Yuma to 32.4 inches at Crown King. Rainfall in excess of 25 inches occurs in a number of locations. The growing season ranges from 348 days in Yuma to less than 100 days at several places in Apache and Coconino Counties.

The sunbelt stereotype is not without basis in fact. Phoenix and Tucson, for example, have sunshine for an average of 86 percent of the possible time. This is compared with 47 percent for Portland, Oregon, 73 percent for Los Angeles, 66 percent for Miami, 59 percent for New York and 57 percent for Chicago.

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Temperatures in the desert plain at a latitude of approximately 32° are frequently higher than temperatures along the equator. Within the state, maximum summer temperatures of 110° to 120° F and minimum winter temperatures of -20° to -30° F are not uncommon. But then, the state also spans a distance of some 400 miles from north to south in addition to the approximately 2½ mile elevational change.

Within 40 miles of Tucson, at Mt. Lemmon, one can traverse five life zones within a time span of about one-half hour, from Lower Sonoran Desert vegetation to fir forest. In the north, within the area bounded by the Painted Desert and the San Francisco Mountains, one can traverse every life zone except the humid tropical. The major vegetation zones in Arizona are desert-shrub, grasslands, chaparral, pinyon-juniper forest and ponderosa pine forest. I will discuss these in a bit more detail later.

### CULTURAL ENVIRONMENT

The Hohokam, Mogollon and Anasazi peoples were among the earliest of Arizonans whose cultures can be documented, at least partially, by physical evidence. The Hohokams, for example, not only constructed dwellings in the Gila and Salt River valleys but also had extensive systems of irrigation canals and dams. This culture disappeared about 1450 A.D., possibly as a result of drought and Apache raids. The Apaches and Navahos probably migrated to Arizona between 1000 and 1300 A.D.

Spanish influence in Arizona can be traced back to the first Spaniard to explore Arizona in 1539, a Franciscan friar named Marcos de Niza. The following years, 1540-42, saw members of Coronado's expedition reach as far north as the Grand Canyon.

During the 17th century attempts were made, with considerable success, to Christianize the Pima, Papago and Moqui Indians. The first presidio was not established, however, until 1752 at Tubac, on the Santa Cruz River just north of what is now Nogales.

American traders and explorers first entered Arizona in the early 19th century. The area of Arizona, north of the Gila River, was brought into the U.S. in 1848 following the Mexican War of 1846. The remainder was acquired through the Gadsden Purchase between 1853 and 1856. It became a separate territory from New Mexico in 1863 and was the last of the lower 48 to achieve statehood in 1912.

Today Arizona has the largest Indian population of any state in the nation. About 5 percent of all Arizonans are Indians in 15 different tribes located on 21 reservations. About one out of every 5.5 Arizonans is of Spanish heritage--19 percent of the state's population. Thus, there are three cultures rooted in the Indian, Spanish and early United States history, each contributing to the social diversity of the state. Obviously, there is also considerable diversity within each of these broad cultural groupings.

## PATTERNS OF GROWTH

Arizona is one of the fastest growing states in the country. It is also the sixth largest state in area, being exceeded in size by Alaska, Texas, California, Montana and New Mexico. With an area of 113,909 square miles, it has a population of approximately 2.3 million and a population density of about 20 people per square mile. A look at the distribution of this population indicates that this is a misleading figure, however, because better than 1.7 million of these people are in metropolitan Phoenix and Tucson. Thus, population density in the rest of the state is closer to 6 people per square mile.

How fast has Arizona grown? The population increased by over 40 percent in the decade 1965 to 1975. Population projections for the period 1970 to 1980 suggest an increase of approximately 50 percent to 2.7 million. For the nation as a whole, however, the projected increase for this time period is around 10 percent a rate of growth that is considerably smaller than that in Arizona.

## RENEWABLE NATURAL RESOURCE MANAGEMENT ISSUES

Much of this growth is occurring in metropolitan Phoenix, within Maricopa County. Maricopa County is also a primary agricultural county. In 1975, 34 percent of all alfalfa, cotton, citrus, grain and vegetable acreage in the state was in Maricopa County (471,740 acres). It is also a primary irrigation area. In 1970, 38 percent of all irrigated lands in the state (430,000 acres) were in this county. The population of Maricopa County has grown from 852,000 in 1965 to 1,218,000 in 1975. Concomitant with this growth is the continuing conversion of substantial amounts of agricultural land, some of the best in the state, to urban uses.

Only 18 percent of the land area in Arizona is privately or corporately owned. Federal ownership accounts for about 42 percent with the major land holding agencies being the Bureau of Land Management (16.7 percent) and the Forest Service (15.9 percent). The balance of the Federal land, about 10 percent, is primarily held by the National Park Service, Fish and Wildlife Service, and Department of Defense. Indian reservations account for another 27 percent and state trust lands account for the remaining 13 percent. Thus, in spite of the size of the state, there is a comparatively limited amount of land available to accommodate continued growth.

It should not be a surprise in a state where 90 percent of the land is classified as arid or semi-arid, that water is the most important renewable natural resource management issue. We use water more rapidly than it can be supplied by nature and the technological efforts of man. There is a dependable annual supply of 2.8 million acre feet and a total annual use of 5 million acre feet. Annually, we are using 2.2 million acre feet more than is replenished. This overdraft is from groundwater.

In Pima County, the county in which Tucson is located, the consumption is 4.7 times the dependable supply resulting in an average lowering of the ground water table by 5.1 feet per year. This is by no means the most extreme case in the state. Rates of depletion are as high as 96 times dependable supply in some of the smaller drainage basins in the state.

Irrigated agriculture is the largest user of water in the state and accounts for 89 percent of the total depletions. The balance is accounted for in municipal and industrial uses, the mining industry and electric power generation.

Given this serious imbalance between dependable supply and annual consumption, it is logical to look to watershed management, in the form of vegetation manipulation, as a means of augmenting available water supplies. Somewhat less than half of the state (36 million acres) is in the desert-shrub vegetation zone, which currently offers little potential for increasing recoverable water supplies. Most of this land is in federal ownership or trusteeship. About one-fourth of the state (18 million acres) is in the grasslands vegetation zone. These lands also offer little potential for increased water yield through vegetation manipulation. The chaparral vegetation zone, covering 8 to 10 percent of the state, does offer some prospects for increasing water yield by the removal of shrub overstories and establishment of grasses and forbs. Experimental work suggests possible increases in some areas of .20 to .50 of an acre foot per acre annually.

The pinyon-juniper woodland zone, comprising about 19 percent of the state and surrounding Arizona's commercial forests, offers little potential for increased water yield through vegetative management. The primary commercial forest is ponderosa pine which covers 7 to 8 percent of the state. It is located primarily along the Mogollon Rim in an unbroken band about 225 miles long — through central Arizona. Experiments on several watersheds with overstory thinning and with different arrangements, orientations and patterns of clearings indicate potential increases in water yield ranging from 16 percent to over 100 percent.

Other vegetation zones include alpine, mixed conifer, aspen and riparian association. Together they comprise less than 1 percent of the area of the state. Of these, experimental data on mixed conifer forests and riparian associations indicate considerable potential for increased water yields.

Each of the vegetation zones which appear to have the greatest potential for increased water yields through vegetation management also have values for other uses including timber production, grazing, recreation and wildlife habitat. Usually these vegetation zones, and in particular the ponderosa pine and riparian associations, are those for which competition among uses is greatest. Thus, managers and researchers are challenged with the task of developing procedures for and facilitating the rational decision processes that take into account the interactive effects of alternative and frequently contrasting management objectives.

The ponderosa pine forest provides a good example of the multiple values and uses that must be considered in the development of management plans. Saw logs are the predominant timber product from Arizona's forests. Of the 363.2 million board feet received at mills, 312 million were ponderosa pine. Of the 24 mills in Arizona, 21 are located in or close to the band of ponderosa pine along the Mogollon Rim.

Ponderosa pine forests are also important habitats for a variety of wildlife including elk, deer, and turkey as well as small game, rodents and game and nongame birds. In a state where most streams are ephemeral, several hundred miles of perennial streams are found in these forests. These streams provide habitat for game and nongame fish as well as water for agricultural, municipal, and industrial uses. It follows quite obviously then, that these forests are also very popular for all forms of recreation, ranging from backpacking to camping, picnicking, hunting, fishing, and all forms of winter sports. This recreation popularity is enhanced by the cooler and more salubrious summer climate which provides welcome relief for residents of the major cities in the hotter temperature zone to the south. Furthermore, much of this land is in public ownership and therefore must be managed to accommodate these multiple uses.

#### SUMMARY

In summary, I have tried to introduce the State of Arizona to you by painting a verbal picture which highlights some of its sharply contrasting features and characteristics. I touched briefly on the contrasts in the physical environment, contrasts in topography, climate and vegetation. The diverse and contrasting cultural environment was presented as a product of the influences and traditions of Arizona's first citizens, the Indians, of several centuries of Spanish influence and of the developments and continued growth associated with incorporation into the United States.

Finally, I reviewed what I perceive to be some of the major renewable natural resources management issues facing the state, including loss of agricultural lands, water depletions, watershed management for increased water yields and the competing and sometimes contrasting demands placed upon our prime commercial forests.

It is, I believe, these contrasts, and a host of others that I have not had time to mention, that make Arizona the attractive and appealing place that it is, and that provide the challenges to natural resources managers and researchers. In concluding, I wish you every success in your meetings which follow and wherein I am sure you will encounter more contrasts (and possibly conflicts) in your deliberations and discussions.

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## DISEASES OF ARID AND SEMI-ARID AREAS

Larry Weir, Moderator

## INTRODUCTION

The title of this panel, as your program tells you, is Diseases of Arid and Semi-Arid Areas. Why I was selected as panel moderator completely escapes me because the topic is one about which I am singularly uninformed. I am, however, happy I was chosen; probably more than I would be if I knew the reason. At least, from the august position of panel moderator there is more of an opportunity to understand what is said because I get a chance to peruse at least those papers I received before the meeting. That way I don't have to either take notes or wait for the proceedings to find out about problems in dry places. Those of you who know me well realize that my association with problems in dry places rarely, if ever, has anything whatsoever to do with diseases.

Let us proceed with the presentations. We have four people on this panel who, unlike their moderator, know about problems in dry places. It seems to me that it has become, over the years, something of a tradition to have at least one paper per meeting that covers diseases peculiar to the meeting area. This tradition is shared this year and the first portion of it will be discussed by Bob Gilbertson. I have no idea why Bob accepted this assignment in view of his attendant problems with local arrangements both for this conference and the up-coming APS meeting. I am, grateful that he did so. Bob will discuss root rots of Southern Arizona Trees.

The second speaker is a personal friend of long-standing. This may, in fact, be the reason that he accepted my request for help. If this is the case, our friendship might possibly be wearing a little thin. Since his retirement he has had little opportunity to continue to be a part of this conference. I am sure that those of you who have been members of this group for some time are as happy as I am to welcome his return. He did pull a fast one on me and decided not to discuss Saguaro as the original program says. His topic at least is applicable to the subject at hand and I'm not about to argue since willing panel members are hard to find. Paul Lightle's topic is, again contrary to the up-to-date program, Some minor forest diseases in Arizona and New Mexico.

The third speaker on the panel needs no introduction to most of you. I appreciate his presence here today because he deliberately jumped into the hole left when Del Wiens decided that going to Africa was the only way he could get out of the assignment I gave him. So instead of his normal topic of dwarf mistletoes, Frank Hawksworth will talk about the big ones.

Our last speaker is Linnae Gillman. I have some, not much, but some sympathy for Linnae. She made the mistake of going to Georgia instead of remaining in Colorado to refuse the task of speaking today. Her place on this panel, while most welcome was assured for her without even asking her until the whole thing was done and she had little choice. Linnae will discuss Black Stain of pinyon pine.

## SOME MINOR FOREST DISEASES IN ARIZONA AND NEW MEXICO

Paul C. Lightle

Dwarfmistletoe and Western Red Rot are, without a doubt, the two greatest causes of wood loss in our southwestern forests. We are so aware of these two bandits that we lose sight of the other things that are slowly but surely robbing us. These small time and occasional pilferers have, for the most part, gone unheeded, indeed scarcely noticed. It is my intention to call some of their names today, to give you a brief description of them, and, where possible, to fill you in on their modus operandi so that you can recognize their nefarious activities the next time your "Dick Tracy" sense tells you that there's something amiss.

Unfortunately, the dossier on most of these small-time criminals is extremely incomplete. For many of them we have only their names. (I won't bother you with their aliases). Some of them do not even have their picture in the rogues' gallery yet, but I must hasten to add that that is our fault.

To begin with the more familiar, let's first discuss needle blight of ponderosa pine.

Most of you are familiar with the symptoms of needle blight. John Staley, Paul Keener, and Stuart Andrews have discussed the disease with you several times, and so I will not bore you by repetition. There are, apparently, four fungi involved in this complex, and together or separately they produce the symptoms. They are Elytroderma deformans, Hypodermella concolor, hacidium spp. and Coryneum cinereum. It takes an expert, or, at least, one who has spent a great deal of time studying the malady, to separate them. In areas where infection persists, mortality, either directly or indirectly attributable to the disease can be expected, and growth of affected trees is markedly reduced.

Next, let us consider the Sooty Bark Canker of aspen caused by Cenangium singulare. Although this disease has only fairly recently been recognized in our area, I feel sure that it has been working "this side of the street" for many years but we failed to recognize that our pockets were being picked. We don't know how serious the losses are, or even the extent of the operation. We do know that it occurs in many places in our aspen stands, and we feel that it may be the most serious of the diseases preying on this timber host. There are at least a couple of other canker diseases of aspen in our region, but they seem to be more spectacular than serious.

The next disease I would like to call to your attention is Atropellis canker of ponderosa pine caused by A. piniphila. It has been present in this region for at least 30 years. It is serious in poles and re-

production on the Lincoln and Mescalero forests in southern New Mexico and on the Prescott in central Arizona. It has not been found on the Kaibab or the Coconino, or the Sitgraves Forests, and has never been seen in northern New Mexico. The loss here is not from mortality or decay but rather from stain. Affected wood is stained steel blue to nearly black. The organism gets started in the axil of a branch and then extends up and down the bole for several feet. While it usually girdles and kills the branch at the infection site, the fungus seldom girdles and bole and so is rarely responsible for mortality. The bark over the cankered area is killed and splits open, and the tree makes a heroic but unsuccessful attempt to callus over the wound. If you cut into the wood under the callus tissue you will find it clean and bright, but if you cut into the wood in the center of the canker, you will find it completely stained, usually so black you can scarcely recognize it was wood. Should this fungus become established and persist in the pulpwood areas of northern Arizona, I feel sure we would be truly alarmed about it. It does not seem to be a threat to sawtimber, but I have seen it in sawlog sized trees on the Mescalero and the Prescott, and I feel sure that affected trees will have to be long-butted, perhaps for half the butt log, when they are harvested.

Red heart caused by Fomes pini is a serious heart rot disease in many places in the United States and elsewhere. However, in our area it is serious in only one location in southern New Mexico. There it causes losses much greater than Western Red Rot. Why this is true we don't know. Since most of you are undoubtedly familiar with red heart, I only mention it in passing.

In somewhat the same category, though occurring more generally through Arizona and New Mexico, are the butt rots caused by Polyporus circinatus and P. schweinitzii. P. circinatus causes a rot that is, in many respects, quite similar to Western Red Rot. In fact, it has been mistaken many times by forest pathologists. However, it doesn't quite look like red rot and has often been labeled "a typical" red rot. It is confined to the stump and butt, seldom extending more than a few feet above the ground.

P. schweinitzii causes a very different type of rot - a brown cubical decay that may extend 6 or 8 feet above the ground, and in a few cases, it will even run through the first log. Both of these fungi fruit infrequently.

The brown cubical rot caused by Fomes officinalis, by contrast, usually enters the tree fairly well up in the crown through a broken limb or a dead or broken top and rather rapidly extends throughout the bole. It fruits rarely and only after the entire stem has been destroyed.

These three heart rots together cause only a small portion of the losses in our forests, and yet they occur frequently enough so that they need to be mentioned as small-time hoods.

Root rots are not causing serious losses in our southwestern forests, still, the mere fact that Fomes annosus and Armillaria mellea are found here, killing occasional trees, is cause for alarm. F. annosus is particularly troublesome in many parts of the world, especially in plantations. It could happen here as we progress from virgin stands to more closely managed forests. Affected trees have the appearance of Ips attacked trees. The tops are often invaded by Ips and many times the damage is diagnosed and treated for Ips beetle attack. Only after repeated attacks do people suspect something other than Ips to be the primary cause. The bark and wood from a foot, or even less, above the ground appears normal and healthy. Indeed it is. Only if the dirt is scraped away at the base and the bark removed, or roots sampled, will you find the real cause of death. In the Southwest, the fungus fruits sparingly in the litter and duff around the base of the tree or even several feet away. The perennial conks are small to tiny usually covered with litter, and are very difficult to see.

In the same category, damage-wise, is the root and butt rot caused by Armillaria mellea. This fungus fruits infrequently but produces mycelial strands and fans which are diagnostic. Resin flow from affected wood may be copious from both of these organisms and so is not particularly useful in diagnosis.

Limb rust caused by Cronartium filamentosum is noted with increasing frequency in our Ponderosa forests. Whether this is because we're beginning to look at the trees instead of just the forest or whether there is actually an increase in diseased trees is difficult to say. The spores of this rust that infect the pines do so through the terminal needles. Infection enters the limb, progresses down the limb to the bole, and then spreads both up and down the bole. The branch originally infected dies about the time the fungus enters the bole and then additional branches die as the tissue around their bases is invaded by the fungus and killed. While the tree may be infected in any portion of the crown, the mid-crown seems to be the area most frequently infected and trees with an upper and a lower crown, but without a mid-crown, may commonly be seen in our forests. The aeciospores produced on the branches and trunk of the pines, mostly in May or June, infect only Indian paintbrush, and the teliospores that infect the pines are produced on the paintbrush beginning about a month after the paintbrush is infected.

There is one more disease that should be mentioned and that is needle rust. The damage from this disease is negligible, at least by current standards, and so practically no work has been done on it. The perfect stage for the rust on ponderosa pine in this area is unknown but all of the needle rusts, when their perfect stages are found, occur in the genus Coleosporium, and we can assume that this fungus will be no exception.

To end this discussion let me mention a group of diseases that have been almost completely ignored. They are the cone and seed diseases. Cone rust occurs occasionally and at times many of the conelets are blighted from this and perhaps other unknown causes. That is the extent of our knowledge. With more emphasis on management, and particularly on regeneration, the need for studies on the diseases affecting the cones and seeds is apparent.

With one exception, this discussion has covered only the robbers that prey on ponderosa pine. This was intentional for two reasons: (1) Ponderosa makes up the great bulk of the volume in our forests, and (2) We know next to nothing about the hoodlums operating in our other coniferous species.

PHORADENDRON IN THE WESTERN UNITED STATES

Frank G. Hawksworth

I'm glad to try to give the Phoradendrons some "equal time"; Phoradendrons have traditionally been the victims of "bad press", or in reality "no press at all". Very little attention has been given to these mistletoes either at W.I.F.D.W.C. or in the forest pathology literature in general. For example, to find the latest list of hosts attacked by various species of Phoradendron, we have to go back more than 60 years to Hedgcock (1915). Dr. Del Wiens and I hope to remedy this situation as we are preparing a manuscript on the taxonomy, hosts, and distribution of Phoradendron in the United States. For this paper, I'll discuss taxonomy, damage, and control of Phoradendron.

## TAXONOMY

Trelease's (1916) monograph of the genus Phoradendron recognized some 240 species including 28 from the United States. However, Trelease's extreme splitting of the genus has not been generally followed and Wien's 1964 treatment of the Acataphyllous (northern) group of Phoradendron recognizes only 12 taxa in the U.S. Also, one outlier of the southern (Cataphyllous) group occurs in southern Florida. The 13 taxa currently recognized in the United States are listed in Table 1. In general, the taxonomy of Phoradendron in the United States has been fairly well worked out although the taxonomic level of some taxa still needs to be determined (e.g., P. bolleanum subsp. densum and pauciflorum, and P. macrophyllum). The major taxonomic problem remaining is the relationship of the eastern P. serotinum (= P. flavescens) to the western P. tomentosum (Wiens 1970). Typical P. serotinum is nearly glabrous while P. tomentosum in southern Texas is densely hairy. Studies are needed in the transition zone between the two taxa in Missouri, Arkansas, and Louisiana, to determine if the differences between the two populations are abrupt or gradual. If the gradation is gradual, the populations might be more appropriately treated as subspecies of the same taxon rather than as separate species.

The distribution of the 11 western Phoradendrons by states is given in Table 2. California and Arizona have the most taxa with 7 each.

The thirteen U.S. Phoradendrons are described briefly. The known principal and rare hosts of the 11 western taxa are given in Appendix I. Any corrections or additions to the host lists would be appreciated.

Table 1. Phoradendron in the United States.

<u>Species</u>	<u>Principal Hosts</u>	<u>Distribution</u>
1. <u>P. bolleanum</u> subsp. <u>densum</u>	Cypress, Junipers	Ore., Calif., Ariz.
2. <u>P. bolleanum</u> subsp. <u>pauciflorum</u>	White fir	Calif., Ariz.
3. <u>P. californicum</u>	Desert legumes	Calif., Nev., Ariz. (leafless) <span style="border: 1px solid black; border-radius: 50%; padding: 2px;">NM</span>
4. <u>P. capitellatum</u>	Junipers	So. Ariz., So. N. Mex.
5. <u>P. hawksworthii</u>	Junipers	So. N. Mex., W. Tex.
6. <u>P. juniperinum</u> subsp. <u>juniperinum</u>	Junipers	Colo. to Ore., south to Mexico. Common leafless species.
7. <u>P. juniperinum</u> subsp. <u>libocedri</u>	Incense cedar	Ore., Calif. (leafless) <span style="border: 1px solid black; border-radius: 50%; padding: 2px;">New?</span>
8. <u>P. rubrum</u>	Mahogany	Southern Florida
9. <u>P. serotinum</u> ( <u>flavescens</u> )	Many hardwoods. Known from about 110 species of 50 genera.	Eastern U.S., N. J., Ohio, Ind., Mo., south to Gulf.
10. <u>P. tomentosum</u>	Hackberry, Mesquite	West Texas, Okla.
11. <u>P. macrophyllum</u>	Many hardwoods--esp. Cottonwood, Ash, and Willow. Known from about 60 species of 30 genera.	West Texas to No. Calif.
12. <u>P. villosum</u> subsp. <u>villosum</u>	Oaks	Calif., Ore.
13. <u>P. villosum</u> subsp. <u>coryae</u>	Oaks	Ariz., N. Mex., Tex.

Table 2. Distribution of Phoradendron in the western United States.

<u>PHORADENDRON</u>	Arizona	California	Colorado	Idaho	Montana	Nevada	New Mexico	Oregon	Texas	Utah	Washington	Wyoming
<u>On Hardwoods</u>												
<u>P. tomentosum</u>									X			
<u>P. macrophyllum</u>	X	X					X		X			
<u>P. villosum</u> subsp. <u>villosum</u>		X						X				
<u>P. villosum</u> subsp. <u>coryae</u>	X						X		X			
<u>P. californicum</u>	X	X				X	X				X	
<u>On Conifers</u>												
<u>P. juniperinum</u> subsp. <u>juniperinum</u>	X	X	X			X	X	X	X	X	X	
<u>P. juniperinum</u> subsp. <u>libocedri</u>		X						X				
<u>P. bolleanum</u> subsp. <u>densum</u>	X	X						X				
<u>P. bolleanum</u> subsp. <u>pauciflorum</u>	X	X										
<u>P. capitellatum</u>	X						X					
<u>P. hawksworthii</u>							X		X			
<u>Phoradendron Totals</u>	7	7	1	0	0	2	6	4	5	2	0	0
<u>Dwarf Mistletoes (Arceuthobium spp.)</u>	8	10	5	5	4	5	6	8	3	6	6	3
<u>MISTLETOE TOTALS</u>	15	17	6	5	4	7	12	12	8	8	6	3

1. PHORADENDRON BOLLEANUM subsp. DENSUM (TORR.) WIENS.--This mistletoe parasitizes junipers and cypress. It has essentially glabrous leaves from 2-3 cm long. Its main range is from the Sierra San Pedro Martir in Baja California, throughout much of California and into southern Oregon, where it attacks many species of Juniperus and Cupressus. An outlier occurs in central Arizona on Cupressus arizonica.
2. PHORADENDRON BOLLEANUM SEEM. subsp. PAUCIFLORUM (TORR.) WIENS - WHITE FIR TRUE MISTLETOE.--This mistletoe is unique in Phoradendron because of its extreme specificity. Its only principal host is white fir, Abies concolor. Its main range is from the Sierra San Pedro Martir in Baja California north to Eldorado County in the central Sierras of California. An extreme disjunct of more than 300 miles occurs in the Santa Catalina Mountains near Tucson.
3. PHORADENDRON CALIFORNICUM NUTT. DESERT MISTLETOE.--The Desert Mistletoe is common on several leguminous desert trees and shrubs including mesquite, palo verde, and acacia in Sonoran deserts. It ranges as far north as southern Nevada and Utah, barely enters southwestern New Mexico, and extends south to northern Sinaloa, Mexico. Its shoots are reddish-brown to yellowish-green and often form large pendulous "bushes" in infected trees. The leaves of the mistletoe are reduced to small scales, making it one of the few leafless true mistletoes. Its pinkish berries mature in winter and are an important food source for many birds. Affected trees do not usually die until they are heavily infected, but this mistletoe is so common in the Southwest that it is considered an important pest problem by many homeowners.
4. PHORADENDRON CAPITELLATUM TORR ex. TREL.--This mistletoe parasitizes junipers. Its geographic range is restricted to central and southeastern Arizona, southwestern New Mexico and northern Chihuahua and Sonora, Mexico. Its leaves are small (1-1.5 cm) and covered with dense stellate hairs. This mistletoe has not been studied extensively, and its distribution and effect on its hosts are poorly known.
5. PHORADENDRON HAWKSWORTHII WIENS (ined).--This juniper mistletoe occurs from southern New Mexico, throughout west Texas, into northern Mexico. The species has been confused with P. capitellatum and P. bolleanum subsp. densum. Its range, in the U.S. at least, apparently doesn't overlap with these other juniper mistletoes. In New Mexico, P. capitellatum occurs only west of the Rio Grande drainage while P. hawksworthii is found to the east of it. This species is characterized by glabrous leaves 1-1.5 cm long and 1-3 mm wide. The taxon has not yet been formally described although it was named in the Flora of Texas (Wiens 1970).

6. PHORADENDRON JUNIPERINUM ENGELM. ex. A. GRAY. JUNIPER MISTLETOE.--Juniper mistletoe occurs on several species of junipers from central Oregon east of the Cascades, south to southern California, eastward through central Nevada to northeastern Utah and west-central Colorado, south to Arizona, New Mexico, western Texas, and into central Mexico. It rarely occurs on cypress (Hawksworth and Wiens 1966). The juniper mistletoe is another example of a true mistletoe whose leaves are reduced to scales. This mistletoe is usually not damaging, but does cause extensive mortality to Utah juniper on the south rim of Grand Canyon National Park (McHenry 1934).
7. PHORADENDRON JUNIPERINUM subsp. LIBOCEDRI (TORR.) WIENS. INCENSE CEDAR MISTLETOE.--This parasite of Incense cedar occurs from the Sierra San Pedro Martir in Baja California, Mexico, north to southern Oregon. It is morphologically similar to P. juniperinum although the shoots tend to be longer and more pendulous.
8. PHORADENDRON RUBRUM (L.) GRISB. MAHOGANY MISTLETOE.--This Caribbean mistletoe occurs in a few localities in the Everglades and on Key Largo in southern Florida (Craighead 1971).
9. PHORADENDRON SEROTINUM (RAF.) M. C. JOHNSTON. EASTERN MISTLETOE.--This, except for P. rubrum in extreme southern Florida, is the only Phoradendron in the East. It ranges from New Jersey, southern Pennsylvania, southern Ohio, southern Indiana, southern Illinois, and southern Missouri, south to the Gulf. It formerly occurred in New York State, but is apparently extinct there now (Britton 1884). The western limits of this taxon and its relationship to the western P. tomentosum are poorly known. This mistletoe has a vast host range of more than 110 species of hardwoods in about 50 genera.
10. PHORADENDRON TOMENTOSUM (D.C.) GRAY.--This mistletoe attacks primarily hackberry and mesquite, but occurs on more than 50 other hosts in Texas and Oklahoma. It has large leaves and is densely hairy. It is the primary species used commercially for Christmas festivities.
11. PHORADENDRON MACROPHYLLUM (ENGELM.) COCKERELL.--This mistletoe parasitizes several hardwood species, especially cottonwood, walnut, ash, and sycamore. Over 30 genera and 60 species of hardwoods have been reported as hosts for this mistletoe. It is distributed from northcentral California along the Coast Ranges and Sierra Nevada foothills into southern Arizona and New Mexico, and south into northern Mexico and western Texas. This mistletoe has been considered to be a subspecies of P. tomentosum (Wiens 1964) but we now regard it as specifically distinct (Hawksworth and Wiens 1979). It is damaging on many orchards and ornamental trees in central California.

12. PHORADENDRON VILLOSUM NUTT. OAK MISTLETOE.--This common mistletoe attacks several species of oaks (and sometimes other trees) from Baja California, through California, north to nearly to Portland, Oregon. It has leaves 2-4 cm wide and is densely hairy with simple hairs.
13. PHORADENDRON VILLOSUM subsp. CORYAE (TREL.) WIENS. SOUTHWESTERN OAK MISTLETOE.--Southwestern oak mistletoe is common on several oaks in the Southwest. It is distributed from central Arizona south into Sonora and Chihuahua, Mexico, west through southern New Mexico and into western Texas. This mistletoe has yellowish-green shoots, small oval leaves with stellate hairs, and produces large white berries which mature in mid-winter.

#### DAMAGE

Although the Phoradendrons are generally not as damaging as the dwarf mistletoes, there are many instances where they are locally serious parasites. The leafy mistletoes are essentially "water parasites" and are less demanding of their hosts than the dwarf mistletoes which are dependent on their hosts for both water and minerals, and other nutrients.

Very little information is available on the effects of Phoradendron on the growth rates of infected trees. However, abnormally high mortality rates associated with heavy mistletoe infection have been reported for several host-parasite combinations, P. juniperinum on Utah juniper at Grand Canyon, Arizona (McHenry 1934), P. macrophyllum on cottonwood in southern New Mexico (Ohmart et al. 1977), P. tomentosum on hackberry in central Texas (Hawksworth, unpublished observations), and P. serotinum on water oak in southern Mississippi (Eleuterius 1976).

Phoradendron bolleanum subsp. pauciflorum is frequently associated with dead tops in white fir in California and Arizona. Not only are vigor of tops affected but, since most white fir cones are produced near the tops of the trees, seed production in affected trees is greatly reduced (Fowells and Schubert 1956, Felix et al. 1971).

#### CONTROL

At present, control of Phoradendron is seldom practiced or needed in forest situations, other than removal of infected trees when practical. However, control is often needed for ornamental trees, where values of individual trees may be high (Johnson 1977, Scharpf and Hawksworth 1974, Walters 1976).

Control is most feasible when infestations are light, once trees become severely infected, control requires more drastic measures and cooperation of adjacent landowners. As all these mistletoes are

evergreens, it is easier to detect them in deciduous hosts during the dormant season. The most effective mistletoe control is to prune out all infected limbs yearly. A safe guide is to cut off the limb 1 foot or more proximal to the mistletoe shoots closest to the bole, preferably at the bole. For mistletoe shoots that occur on the main stem, control may be practiced by (1) merely knocking off the shoots every 1 or 2 years, or (2) by knocking off the shoots and wrapping the affected area with a band of black polyethylene wide enough to exclude light, and tying it tightly with twine or tape. If the plastic is left in place for a year or so, the root system of the mistletoe is said to be killed (Johnson 1977). However, others have questioned the efficiency of this method so further tests are needed.

Several herbicides, mainly 2,4-D and similar compounds are effective against Phoradendron in walnut orchards in California (Bayer et al. 1957, Graser 1954) and in pecan in Florida (French 1970).

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APPENDIX I. HOSTS OF PHORADENDRON IN THE WESTERN UNITED STATES

PHORADENORON	COMMON HOSTS	RARE HOSTS
<i>P. bolleanum</i> subsp. <i>densum</i>	<i>Cupressus arizonica</i> <i>Cupressus bakeri</i> <i>Cupressus goveniana</i> <i>Cupressus macnabiana</i> <i>Cupressus sargentii</i> <i>Juniperus californica</i> <i>Juniperus occidentalis</i> <i>Juniperus osteosperma</i>	<i>Cupressus macrocarpa</i> <i>Pinus monophylla</i>
<i>P. bolleanum</i> subsp. <i>pauciflorum</i>	<i>Abies concolor</i>	<i>Phoradendron bolleanum</i> subsp. <i>pauciflorum</i>
<i>P. californicum</i>	<i>Acacia constricta</i> <i>Acacia farnesiana</i> <i>Acacia greggii</i> <i>Acacia millefolia</i> <i>Cercidium floridum</i> <i>Cercidium microphyllum</i> <i>Dalea spinosa</i> <i>Olneya tesota</i> <i>Parkinsonia aculeata</i> <i>Prosopis juliflora</i> <i>Prosopis pubescens</i>	<i>Baccharis viminea</i> <i>Calliandra humilis</i> <i>Ceanothus</i> sp. <i>Condalia lycioides</i> <i>Condalia mexicana</i> <i>Condalia spathulata</i> <i>Foquieria splendens</i> <i>Frazinus</i> sp. <i>Larrea divaricata</i> <i>Phoradendron californicum</i> <i>Phoradendron macrophyllum</i> <i>Pithecellobium confine</i> <i>Simmondsia chinensis</i> <i>Tamarix</i> sp.
<i>P. capitellatum</i>	<i>Juniperus deppeana</i> <i>Juniperus erythrocarpa</i> <i>Juniperus monosperma</i> <i>Juniperus osteosperma</i>	
<i>P. hawksworthii</i>	<i>Juniperus ashei</i> <i>Juniperus deppeana</i> <i>Juniperus erythrocarpa</i> <i>Juniperus flaccida</i> <i>Juniperus monosperma</i> <i>Juniperus pinchotii</i>	
<i>P. juniperinum</i> subsp. <i>juniperinum</i>	<i>Juniperus californica</i> <i>Juniperus deppeana</i> <i>Juniperus erythrocarpa</i> <i>Juniperus flaccida</i> <i>Juniperus monosperma</i> <i>Juniperus occidentalis</i> <i>Juniperus osteosperma</i> <i>Juniperus scopulorum</i>	<i>Chamaebatiaria millifolium</i> <i>Cupressus arizonica</i> <i>Cupressus bakeri</i> <i>Pinus monophylla</i>

## PHORADENDRON

*P. juniperinum* subsp.  
*liboedri*

*P. tomentosum*

(Note: The distribution of this taxon and the eastern *P. serotinum* (= *P. flavescens*) are not yet understood. Only the hosts in west Texas are listed here).

*P. macrophyllum*

## COMMON HOSTS

*Libocedrus decurrens*

*Acacia greggii*  
*Celtis reticulata*  
*Prosopis juliflora*

*Fraxinus cuspidata*  
*Fraxinus dipetala*  
*Fraxinus latifolia*  
*Fraxinus valutina*  
*Juglans californica*  
*Juglans hindsii*  
*Juglans major*  
*Platanus racemosa*  
*Platanus wrightii*  
*Populus fremontii*  
*Salix exigua*  
*Salix fluviatilis*  
*Salix gooddingii*  
*Salix laevigata*  
*Salix lasiolepis*  
*Salix nigra*  
*Salix taxifolia*

## RARE HOSTS

*Chamaecyparis lawsoniana*  
*Phoradendron bolleanum*  
subsp. *pauciflorum*

*Fraxinus* sp.  
*Gleditsia triacanthos*  
*Juglans microcarpa*  
*Quercus gravesii*  
*Robinia pseudoacacia*

*Acer macrophyllum*  
*Acer negundo*  
*Acer saccharinum*  
*Aesculus californica*  
*Alnus oblongifolia*  
*Alnus rhombifolia*  
*Betula* sp.  
*Carya pecan*  
*Celtis australis*  
*Celtis reticulata*  
*Celtis sinensis*  
*Cytisus proliferus*  
*Diospyros kaki*  
*Diospyros lotus*  
*Diospyros virginiana*  
*Eriogonum pauciflorum*  
*Forestiera* sp.  
*Fraxinus americana*  
*Gleditsia triacanthos*  
*Juglans nigra*  
*Juglans regia*  
*Maclura pomifera*  
*Malus sylvestris*  
*Nicotiana glauca*  
*Platanus acerifolia*  
*Populus angustifolia*  
*Populus deltoides*  
*Populus nigra*  
*Populus trichocarpa*  
*Prosopis juliflora*  
*Prosopis pubescens*  
*Prunus* sp.  
*Punica granatum*  
*Pyrus communis*  
*Robinia pseudoacacia*  
*Salix babylonica*  
*Sapindus drummondii*  
*Ulmus americana*  
*Ulmus parvifolia*  
*Ulmus pumila*  
*Umbellaria californica*  
*Zelkova serrata*

PHORADENDRON

COMMON HOSTS

RARE HOSTS

*P. villosum* subsp. *villosum*

*Quercus agrifolia*  
*Quercus chrysolepis*  
*Quercus douglasii*  
*Quercus dumosa*  
*Quercus durata*  
*Quercus garryana*  
*Quercus kelloggii*  
*Quercus lobata*  
*Quercus Xmoreha*  
*Quercus turbinella*  
*Quercus wislizenii*

*Acacia* sp.  
*Adenostoma fasciculatum*  
*Aesculus californica*  
*Alnus rhombifolia*  
*Arctostaphylos manzanita*  
*Arctostaphylos patula*  
*Castanea dentata*  
*Castaniopsis sempervirens*  
*Ceanothus cuneatus*  
*Cercocarpus betuloides*  
*Cercocarpus brevifolius*  
*Fraxinus* sp.  
*Lithocarpus densiflora*  
*Phoradendron bolleanum*  
 subsp. *pauciflorum*  
*Phoradendron villosum*  
*Populus nigra*  
*Prunus persica*  
*Rhus diversiloba*  
*Rhus integrifolia*  
*Robinia pseudoacacia*  
*Salix* sp.  
*Umbellularia californica*

*P. villosum* subsp. *coryae*

*Quercus arizonica*  
*Quercus chrysolepis*  
*Quercus dumii*  
*Quercus emoryi*  
*Quercus gambelii*  
*Quercus gravesii*  
*Quercus grisea*  
*Quercus hypoleucoides*  
*Quercus oblongifolia*  
*Quercus pungens*  
*Quercus reticulata*  
*Quercus towneyi*  
*Quercus turbinella*  
*Quercus undulata*

*Acacia amentacea*  
*Berberis nevadensis*  
*Celtis reticulata*  
*Condalia globosa*  
*Forestiera neomexicana*  
*Fraxinus velutina*  
*Juglans major*  
*Phoradendron juniperinum*  
*Populus* sp.  
*Prosopis* sp.  
*Vauquelinia californica*

## BLACK STAIN ROOT DISEASE IN COLORADO

Linnea S. Gillman

### SUMMARY

Verticicladiella wagnerii, a pathogen on pines and Douglas-fir in the western United States, is causing mortality of pinyon in western Colorado. Forest Insect and Disease Management (USDA Forest Service) staff members, in cooperation with Mesa Verde National Park and the Colorado State Forest Service, have been investigating the problem. We performed aerial and ground surveys in 1975 to determine the geographic distribution of the disease, and in 1978 we began an evaluation in Mesa Verde National Park to determine the effect of V. wagnerii on pinyon.

### 1975 COLORADO SURVEY

Groups of dead and dying pinyon were mapped from the air throughout the pinyon-juniper type. Stands with possible disease centers were checked on the ground by sampling the roots and root collar. Those with the typical black stain were taken to the laboratory for confirmation of black stain root by cultural isolations.

The disease was found to be present throughout the range of pinyon west of the Continental Divide, but was not detected in any trees in eastern Colorado. So far, the Divide appears to be a barrier to the eastern spread of the disease.

One disease center was excavated, revealing numerous root contacts and several grafts through which the fungus had apparently spread.

In Colorado, the disease occurred on the better pinyon sites, those with cooler and more moist environments. This observation is in agreement with studies in California in which the disease was more common on sites with high soil moisture.

### MESA VERDE NATIONAL PARK

Mesa Verde is a 52,000 acre National Park in the southwestern corner of Colorado, with elevations of 6,500' to 8,500 feet. It is a flat tableland dissected by deep canyons into somewhat isolated areas, called mesas. The Park's climate is classified as a cold, middle latitude, semiarid one. Average yearly precipitation is 18 inches.

The Park is in the pinyon-juniper climax region, and the trees grow taller and closer together than in the type region generally. In the higher parts of the mesa, fire plays an important role in controlling the vegetation type. Succession after a fire results in a dominance of brush after 25 years, and a thicket after about 100 years. The pinyon-juniper type gradually takes over after several hundred years. The present policy of fire suppression is gradually resulting in the pinyon-juniper forest replacing the former extensive shrub vegetation.

The Park is presently concerned about the many dead and dying pinyon that are throughout the area. This decline was first confirmed as the black stain root disease by J. L. Mielke in 1942. In 1933 and 1934, 12,000 dead and dying pinyon in the park were cut and burned, and stump sampling 10 years later showed the typical stain of the disease in a large proportion of the trees. This disease has undoubtedly been in the park for a long time.

The pinyon-juniper stands in Mesa Verde are approaching climax, but the structure is not typical of a climax stand. There is a lack of old pinyon and an abundance of much older juniper. This suggests that some factor has adversely affected the pinyon component of the stands. One possibility is the black stain root disease, which we have observed to be predominantly killing mature trees. Wagener and Mielke observed that losses of mature trees have been heavy, but small trees with limited root systems escape and form the nucleus of a new stand. The juniper is unaffected by the disease. Another factor which may have caused the youthful pinyon stands is a severe drought that occurred in the southwest several centuries ago that caused much die-out of less resistant tree species.

Our current investigation of the pinyon mortality problem in Mesa Verde began in May 1978 with aerial reconnaissance. In a total of 28,000 acres of pinyon-juniper type mapped, there were approximately 175 disease centers that contained pinyon with red foliage. The centers varied in size from one tree to many acres each. The centers were located throughout the park, including high visitor use areas. Ground checks of many centers confirmed the presence of black stain root disease in every center of dead and dying pinyon.

Our next activity was a reinvestigation of a permanent plot set up by Mielke in 1944. He marked 73 trees, of which 5 were dead of V. wagnerii. Mielke followed the plot annually over eight years, and during this time the disease killed an additional 28 trees and spread a distance of 38 feet. The next count after 1952 was in 1972, when there were 56 dead trees, an average of a little over one per year. No new mortality has occurred between 1972 and 1978. This center was apparently very active in the 1940's, but now has stabilized. The abundant younger pinyon reproduction is so far untouched by disease, and we have mapped the reproduction to follow its progress.

Our observations in the Park indicate that centers are active for a time and then the disease apparently slows down or stops. The assumption is that the undiseased reproduction then establishes a new pinyon stand, since the fungus is not a saprophyte in the remaining dead stumps. In order to better understand the spread of the disease, we have established permanent plots in disease centers in several locations throughout the park. New mortality will be mapped over the years, and we will be able to plot the rate of spread and longevity of a center in a variety of habitats.

THE CURIOUS ANATOMY OF ARCEUTHOBIUM IN REGARDS  
TO HOST/PARASITE WATER RELATIONS AND TRANSLOCATION

M. Carol Alosi<sup>1</sup>

INTRODUCTION

One of the most helpful concepts to use when dealing with the interaction of plant structure and function is the apoplast/symplast concept (9). This concept compartmentalizes the total plant body into a non-living component of cell walls and intercellular spaces--the apoplast; and a living cytoplasmic component--the symplast. Components can be dealt with as independent continuous systems isolated from each other but still interacting by virtue of the unique properties of the plasmalemma. Molecules, ions and microscopic pathogens may move about or be concentrated in quite different manners depending on whether they are apoplastically or symplastically held. The protoplasm of the symplast is interconnected by tiny cytoplasmic channels through the cell walls. These channels, which join adjacent cells are termed plasmodesmata. Thus, sugars and metabolites may move from cell to cell through these channels without leaving the symplast, while other substances such as water and minerals may travel for long distances through the free space of the plant body apoplast without leaving cell walls or intercellular spaces to enter a living cell. Despite their independency, the apoplast and symplast still function in a state of dynamic equilibrium with each other since water--a sort of thermodynamic equilibrating factor--moves freely across the plasmalemma from one compartment to another (9).

When a plant becomes infected with an obligate parasite, apoplast/symplast compartmentalization becomes more complex since a duality of systems is involved. However, the physiology of the host/parasite relationship can perhaps be more meaningfully understood when the physical relationships of host's and parasite's apoplasts and symplasts are known. For example, the water and mineral parasite may integrate its apoplast with the host apoplast in such a manner as to form a common apoplast. With no apoplastic barriers isolating the organisms, the flow of water and nutrients can be a passive process governed by thermodynamic laws. The holoparasite must not only channel apoplastic flow into parasite regions of the common apoplast, but must also affect translocation in the host symplast so that symplastically mobile nutrients are made available to the parasite.

OBSERVATIONS AND DISCUSSION

The success of an obligate parasite might be judged on how effectively the parasite can acquire nutrients while still allowing the host to maintain a level of vigor supportive to the continual presence of the parasite. Based on these criteria the dwarf mistletoe, Arceuthobium, is

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a holoparasite of coniferous trees that appears to be a very successful obligate parasite. It integrates its endophytic system with host vascular tissue in such a manner so as to remain associated with nutrient-rich host tissue for many years. This association creates a minimal amount of disruption of normal host tissue. The unique tissue arrangement is achieved through the establishment of meristematic parasite tissue in the host cambial zone (11). Within the cambium, parasite tissue becomes associated with host ray initials and derivative production to the xylem and phloem sides of the cambium is coordinated with host tissues. Fig. 1 illustrates the relationship of endophytic tissue with the host stem. Eventually parasite tissue becomes so successfully incorporated into what appear to be multiseriate rays that, without special histochemical techniques or phase contrast microscope optics, one sometimes cannot tell host cells from parasite cells.

In both light and electron microscope studies the mistletoe cell walls often appear fused with the surrounding host ray cell walls and pit-like regions are seen in the adjoining walls. The light microscope does not allow one to determine whether or not plasmatic connections between host and parasite exist in regions of wall thinnings. But electron micrographs of thin wall regions between hemlock cells and parasite cells shows that, although the wall thins to only a fraction of a micron, there is no membrane fusion between the two organisms in this area. In some pine-Arceuthobium associations, there is a tendency for half plasmodesmata to be formed on the pine side of the contiguous walls; but the plasmodesmata end blindly without joining with the dwarf mistletoe protoplast. An extensive light and electron microscope investigation has allowed me to conclude that Arceuthobium-infected tissue can be considered in terms of three compartments: 1) a living continuum of parasite protoplasm - the parasite symplast; 2) a living continuum of host protoplasm - the host symplast; 3) and a common apoplast.

The apoplastic continuum between host and parasite allows direct access to apoplastically mobile nutrients. But each of the organisms' symplasts appear to remain distinct.

Arceuthobium obtains substantial amounts of host-originating photosynthate (5,8). Photosynthate is symplastically translocated (2); but since Arceuthobium does not appear to have direct access to these nutrients through interspecific symplastic continuities, the only way Arceuthobium could accumulate products of host photosynthesis is by leakage of these host symplastically held nutrients into the common apoplast. Nutrients may then be distributed passively and apoplastically within parasite cell walls. Additionally, nutrients may be actively loaded into the parasite symplast through membrane-mediated events.

As far as is known there is no specialized tissue (such as phloem) in Arceuthobium that functions to concentrate and rapidly move carbon-based nutrients acquired from the host to other regions of the parasite symplast.

Thus, it must be assumed that nutrients are distributed through the parasite symplast by diffusion through plasmodesmata. The rate of diffusion will be influenced by protoplasmic streaming and/or a symplastic pressure gradient. The presence of a symplastic pressure gradient is suggested by differential distribution of starch through the parasite plant body. Starch is not found in the most of the endophytic system, but is stored in the aerial shoots. This situation is the reverse of that found in most plants, where starch is heavily stored in the roots, with lesser amounts in the aerial portions. Starch synthesis, it will be recalled, lowers the osmotic concentration of the cell by removing dissolved sugars from the cytosol. Therefore, lack of starch stores in the endophytic system near the source of osmotically active carbon-based nutrients will intensify a solute gradient toward the aerial shoots--where solute concentrations are reduced by polymerizing sugars into insoluble starch stores.

Differential starch storage within the parasite symplast may enhance the rate of diffusion of solutes through the parasite plant body, but it is likely that there are other ways for the parasite to make nutrient acquisition and translocation efficient. Some investigators have proposed the existence of a phloetracheid system in certain parasitic angiosperms devoid of phloem (1,7). This term implies that the xylem may facilitate the translocation of carbon-based nutrients in addition to normal tracheary nutrients. However, it is difficult to see how sugars or other host-originating nutrients could be concentrated selectively in the xylem since no apoplastic barriers (such as the casparian strips of roots) were found. It seems more plausible that carbon solutes leaked from the host symplast are distributed throughout the whole of the apoplastic region associated with endophytic tissue and further through the apoplastic continuum into the aerial shoots.

A problem to consider in developing this hypothesis is: how could nutrients be preferentially distributed to apoplast regions around Arceuthobium cells and then passively translocated into the aerial shoots?

To answer this question we must consider water potential concepts. Water flows "downhill" from a region of high water potential to lower potential, in order to equilibrate different regions of a continuous system. Substances dissolved in the water may be flushed along with the water provided there are no barriers to the solutes along the pathway. The simplest formula to obtain a value of water potential ( $\psi$ ) at a given region in a system is given by:  $\psi = P - \pi$  (13). Where P is usually thought of as a mechanical pressure such as turgor pressure and  $\pi$  is the solute concentration expressed as osmotic pressure. P in the apoplast often has a negative value. This is due to the tension or pull on the system that is generated by evaporation at air/water interfaces. During periods of transpiration the closer to the regions of evaporation (stomates, cuticles, intercellular spaces in leaves) the lower the water potential due to the increasingly negative P.

Scholander, et al (10) using the pressure bomb apparatus he designed, compared water potential values of host and parasite and determined that the parasites he tested always had significantly lower water potential values. Before Scholander, other investigators have demonstrated that some parasitic angiosperms have higher transpiration rates than their hosts under certain conditions (4,6,12).

Recently Fisher (3) studied water relations of dwarf mistletoe on pine under various conditions of water stress. His results show that Arceuthobium is capable of generating significantly lower water potential in its aerial shoots as compared to host branches. The results given in his thesis abstract seemed to indicate that high transpiration rates of the parasite were responsible for the low water potential.

Since transpiration rates are strongly a function of the leaf or shoot anatomy (9) it is very disturbing to try to correlate Fisher's physiological measurements with dwarf mistletoe aerial shoot anatomy. The stem and leaf of Arceuthobium appear to be designed to produce a high resistance to transpiration rather than to promote transpiration.

Aerial shoots of Arceuthobium appear leafless and exhibit a low surface to volume ratio characteristic of xerophytic or drought tolerant plants. Close examination of shoots shows that Arceuthobium does have leaves but they are small structures cupped around the nodes. A cross section through one of the small Arceuthobium leaves reveals many more modifications that may reduce transpiration rates (Fig. 2).

1. Note the closely packed cells. There is no arrangement into the typical dicotyledonous leaf structure of spongy mesophyll and palisade tissue.
2. Also note the almost complete lack of a sub-stomatal chamber subtending the guard cells. This feature, like the small amount of intercellular space throughout the leaf reduces the evaporative surfaces from the apoplast and limits the leaf's ability to generate high saturated vapor pressure values and apoplastic tension. These factors in turn would reduce the rate of transpiration through the stomata.
3. The guard cells are partially covered by over-arching accessory cells to produce a small antechamber just above the stomatal aperture. This feature enlarges the boundary layer of "dead" space around the pore and reduces the effect of convection on transpiration.
4. Finally, note the thick cuticle of the Arceuthobium leaf, a modification that reduces water loss from evaporation of water from the epidermis.

These features of Arceuthobium leaf anatomy--low surface to volume ratio, little intercellular space, lack of a substantial substomatal chamber, sunken guard cells, and thick cuticle--do not support the conclusion, based on the available physiological data, that Arceuthobium maintains low water potentials within aerial shoots solely by high transpiration rates.

But is there another method reconcilable with parasite anatomy that might account for the parasite's ability to mobilize water and nutrients into the aerial shoots?

It will be recalled that the only thermodynamically-acceptable way for water and nutrients to flow preferentially into parasite regions of the host/parasite apoplast, and subsequently out into the aerial shoot portions of the apoplast, is by the existence of a water potential gradient between the court of the infection and the aerial shoots.

In the water potential formula given earlier  $\psi = P - \pi$ , a negative  $P$  value is an indication of tension in the system due to transpiration, and  $\pi$  is an indication of the solute concentration. Because of the contribution of  $\pi$ , it is not necessary for  $P_{\text{parasite}}$  to be more negative (indicative of higher transpiration rates) than  $P_{\text{host}}$  in order to generate a low water potential, as long as  $\pi_{\text{parasite}}$  is greater than  $\pi_{\text{host}}$ . Thus, apoplastic solutes ( $\pi$ ) in the parasite regions may be a contributing factor to the generation of water potential differences between parasite and host.

I see the possibility of a connection between the ability to lower water potential values by apoplastic solutes and the lack of phloem tissue in Arceuthobium and other parasitic angiosperms:

Phloem is a solute concentrating tissue. If host-originating solutes were removed from the apoplast, concentrated in phloem, and then stored in insoluble forms, the ability to generate low water potentials in the apoplast by solutes may be diminished. But, by allowing relatively high solute concentrations to persist in the apoplast, the parasite is able to utilize these solutes to steepen water potential gradients between host and parasite tissues. A dual system, utilizing both apoplastic solutes and transpiration would allow the parasite to maintain a competitive versatility under various environmental conditions.

These hypotheses have been generated by my conviction that plant structure is indicative of function. Where structure does not appear to support conclusions based on quantitative data, it may be necessary to reinvestigate the problem to try to reconcile an apparent conflict in structure and function.

Fisher compared host and parasite transpiration rates under stress conditions using the cuvette/psychrometer method for determining water loss.

Perhaps the potometer method, where cut stems are supplied pure water might allow different interpretations. Also, it would be interesting to know if under conditions of near zero transpiration, whether parasite aerial shoots could still maintain lower water potentials than the host stem. Another area for investigation that might clarify aspects of Arceuthobium nutrition is microautoradiographical study of soluble substances to try to determine if host originating carbon compounds are, indeed, distributed through the parasite's apoplast.

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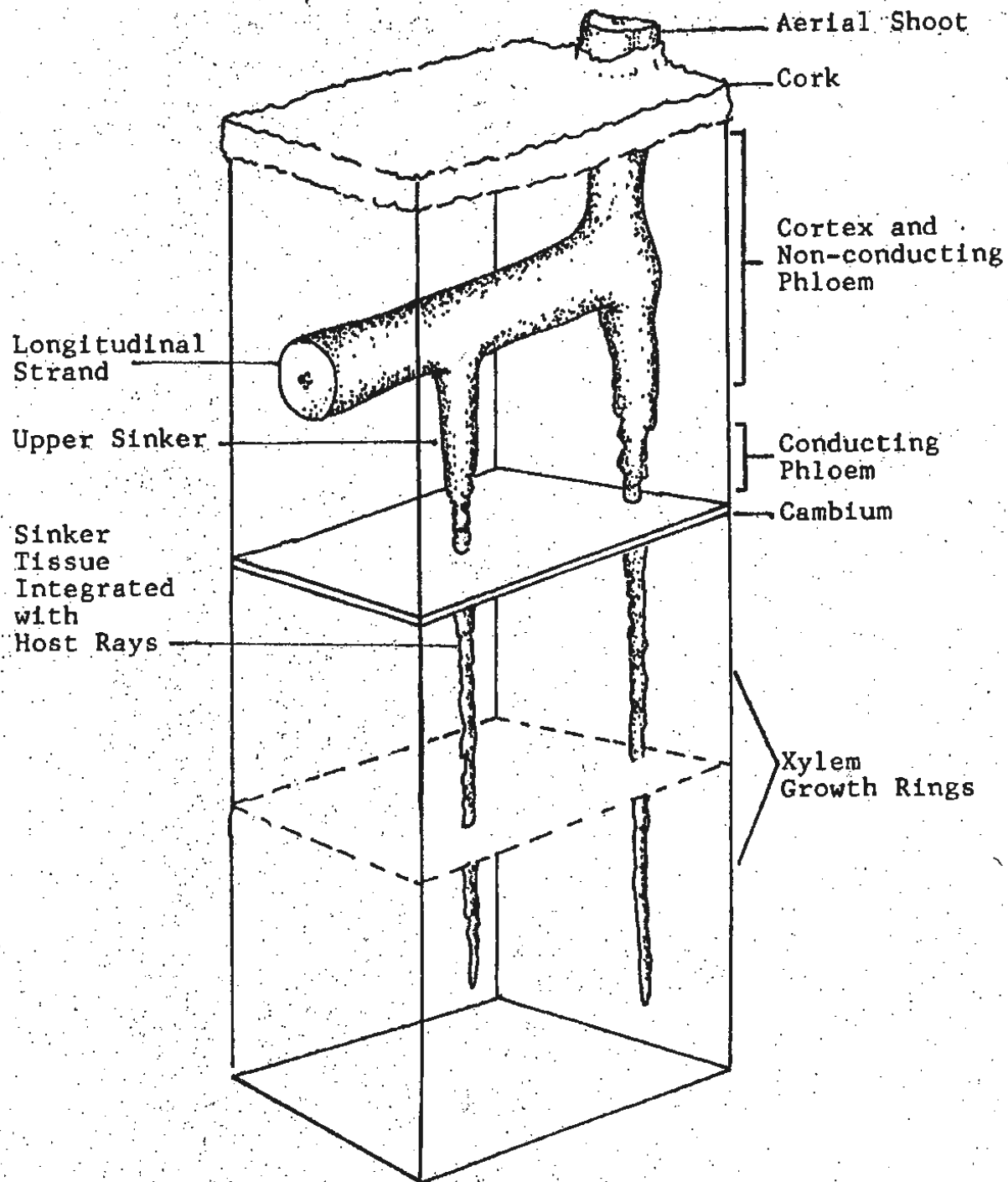


Figure 1. Three dimensional diagram of the relationships of the endophytic tissue of Arcēuthobium to host stem tissue. Parasite tissue is stippled.

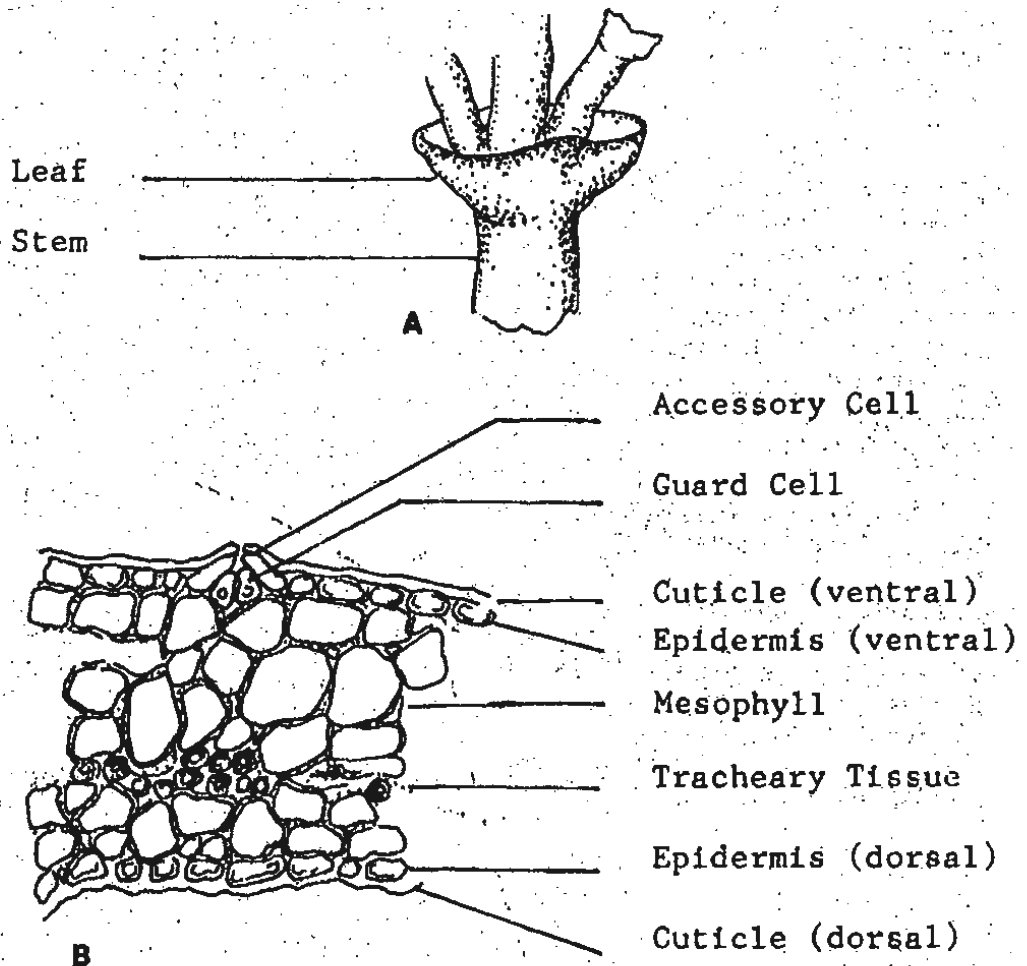


Figure 2. A. Drawing of a portion of the aerial shoot of Arceuthobium tsugense to show the relationship of the much-reduced leaves to the stem. 8X.  
 B. Cross section of Arceuthobium leaf which reveals certain xerophytic modifications (see text).

HOST-PARASITE INTERACTIONS OF CRONARTIUM  
RIBICOLA AND PINUS MONTICOLA

Neil E. Martin

Cronartium ribicola is a macrocyclic heteroecious rust that causes the blister rust disease of five needled pines. This fungus produces haustoria in both ribes and pine hosts that differ in appearance and anatomy.

Hauustoria of C. ribicola in pine bark are extensions of the haploid mycelium into parenchyma or parenchyma-like cells of the bark and rays. These haustoria are clavate, of consistent radius along the long axis, and constricted at the point of penetration of the host cell wall. They can develop into elongated, branching, coiling or twisting structures having the morphology of hyphae. Transmission electron microscope aided observations of longitudinal sections of haustoria found the haploid stage haustorium to be similar to the intercellular hyphae. The capsular sheath and the wall of the mother cell are continuous with that of the haustorium. The haustorium does not have a neck distinct from its body but is constricted at the point of passage through the host cell wall. Neck bands are absent, but collars of host cell wall material are frequent.

Hauustoria of the n+n stage found in ribes are morphologically different from their associated mycelium and from the haploid stage haustoria and mycelium in pine. The haustoria mother cell is thickened in the area of contact with the host cell. The walls of the haustoria mother cell are not continuous through the passage into the host cell. The haustoria are saccate, without septa, have a neck distinct from the body, and neck bands are evident.

The intercellular hyphae of both nuclear stages are affixed to host cell wall by a substance(s) that can be observed with the aid of transmission and scanning electron microscopes. It is not known if this substance(s) is pectin, but it is assumed that pectin and the products of pectin catabolism are encountered by both host and pathogen cells early in the penetration process. Western white pine bark parenchyma cells and mycelium isolated from branch cankers were challenged with pectins on artificial media. The methyl esterases are perhaps the first to act upon the pectin to expose host and pathogen to a low methoxy pectin compound similar to citrus pectin. Continued enzymatic activity likely produces a compound void of methyl groups which can be represented by pectic acid. Pine cells rapidly became brown and died in 0.1 percent citrus pectin and 0.05 percent pectic acid. These treatments were fungistatic to the rust fungus and symptoms of death such as lysis or color change were not evident. Polygalacturonic acid and sodium polypectate had little influence on either organism. Growth response by both organisms was within the variation within treatments. It was apparent that the rust fungus could tolerate compounds and concentrations that the host cells could

not, which supports the basis for hypersensitivity, a common resistance reaction.

Climate effects changes in the host-parasite interaction that can be measured in the pool of soluble sugars. The seasons of the temperate zone trigger distinct periods of growth and dormancy in western white pine. Amounts of sugars in needles and bark reflect these periods by being depleted by growth and stored during dormancy. Sugars are also used by the rust fungus within and near bole cankers, but are not influenced to be transported great distances from needles. Climate and rust fungus interact differently than climate and pine. Although aeciospores are disseminated in early spring, the physiology of the fungus is active in the winter months whenever the bark temperatures exceed the growth threshold value for the rust. In February, a marked depletion in amounts of soluble sugars was found in areas of cankers that produce aeciospores. Similar but lesser effects were measured in bark tissues adjacent to the blister rust canker margin. Although chemical control of this rust is not in the immediate future, future studies may profitably exploit such differences in physiology.

High level resistance in host-parasite interactions is the best and most desirable solution to the blister rust problem of five needled pines. However, many low-level resistance mechanisms exist and when their effects are simulated in a computer model their utility is apparent. The low-level resistance mechanisms of reduced needle lesions, slow growth of the rust in branches and boles, and retention of needles for only 2 years when combined would yield 1 percent mortality from blister rust in 100 years.

Rating the hazard of a site through distribution mapping of ribes bushes and site descriptions would provide a risk rating for the land manager. Ribes eradication before planting is a feasible and profitable investment in site preparation. Fertilization and other cultural techniques that encourage growth when coupled with breeding for rapid growth will help five needled pines to pass through their most vulnerable stage quickly. If unexpectedly high levels of potentially lethal infections are found, the branches having these cankers can be removed via pruning.

Integration of certain or all resistance mechanisms, cultural treatments, biological controls, and chemical controls becomes closer to reality as these and other host-parasite relationships are elucidated.

SEM AND LIGHT MICROSCOPY OF INFECTION OF  
PINUS NIGRA AND P. RADIATA BY DOTHISTROMA PINI

Robert F. Patton and Russell N. Spear<sup>1</sup>

Up to the present time our knowledge of details of infection of pine needles by Dothistroma pini has come from the work of Gadgil in New Zealand, Ivory in East Africa, Peterson in Nebraska, and most recently, Muir in California, whose Ph.D. thesis (1974) has not been published. Their results do not agree in all respects, and the differences may be those between 1) field and growth room conditions, 2) natural and artificial inoculations, 3) hosts, and 4) varieties of the fungus. In order to clarify and expand upon details of the infection process in Dothistroma needle blight, we applied scanning electron microscopy in observing naturally-infected needles of Pinus nigra from Wisconsin and P. radiata from New Zealand, and supplemented these observations with light microscopy of sectional specimens.

Germination of Conidia and Directed Germ Tube Growth

For germination of conidia temperature is generally not a limiting factor. Conidia germinate at a range of temperatures between about 8°-28°C. with optima falling within the range of 18°-24° C. (Ivory 1967, Gadgil 1967, Sheridan and Yen 1970, Peterson 1967).

Moisture is necessary for germination, but it is not entirely clear whether a film of free water is required. Most workers indicated liquid water is necessary (Ivory 1967, Gadgil 1967, Gibson 1972). But Sheridan and Yen (1970) in New Zealand obtained good germination at 98-100% relative humidity, and there was the possibility of some germination at a relative humidity as low as 76%, although this might have resulted from a carry-over of imbibed moisture when conidia were in suspension. Also, Ivory (1967) and Gibson et al (1964) indicated that trace amounts of nitrogenous nutrients were also essential.

Information on time and character of germination in vitro was reported by Gadgil (1967). He obtained 70% germination after 48 hours, and 90% after three days. Usually spores formed 1-3 germ tubes, and anastomosis between germ tubes was common.

Peterson (1967, 1969) investigated germ tube growth by examining plastic leaf impressions of needles of Austrian (P. nigra) and Ponderosa (P. ponderosa) pines naturally infected with D. pini var. pini. Germ tube growth was positively directed toward stomata - more than 80% of observed germ tubes grew toward stomata. They often abruptly changed direction of growth toward stomata, frequently as much as 90 degrees. He reported no additional details of the infection process, and instead placed emphasis on variation in susceptibility of needles according to

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their age. Neither Gadgil (1967) nor Ivory (1972) confirmed Peterson's observations of a marked effect of stomata on germ tube growth. Peterson's observations were of field material where moisture gradients were possible, whereas Gadgil and Ivory used saturated atmospheres, and moisture or other gaseous gradients, if they were important, would not be present. Gadgil (1967) sprayed P. radiata seedlings with conidial suspensions or a suspension of a blended agar culture of mycelium and spores of D. pini var. pini and incubated trees in a moist chamber. He obtained profuse growth of surface mycelium and hyphae grew at random. Under these conditions stomata exerted no special attraction.

Muir (1974) observed similar extensive growth of hyphae on needles of artificially inoculated P. radiata seedlings (presumably with D. pini var. linearis) incubated in growth chambers. His observations of germ tube growth on needles naturally infected in the field, however, supported Peterson's observations of directed growth. Muir's observations suggested to him that the stimulus occurred periodically.

Our direct observations by SEM of germ tube growth both of Scirrhia acicola conidia on needles of Scots pine (P. sylvestris), and of D. pini var. pini conidia on needles of Austrian pine, do indeed indicate that under natural conditions in the field germ tubes of these fungi respond to some stimulus from stomata and grow toward them. We agree with Muir that the stimulus varies with time or with individual stomata.

#### Entry into Stomata and Development in Stomatal Antechamber

There is general agreement that infection occurs by penetration through stomata, but details of the process vary according to the investigator.

Gadgil's (1967) artificial inoculations resulted in profuse mycelial growth on the surface of needles of P. radiata seedlings incubated in a moist chamber. The mycelium formed massive clumps over stomatal openings and filled the stomatal antechamber. A narrow hypha penetrated between the guard cells and branched out into the substomatal chamber and adjacent mesophyll. Such examples of penetration were seen only rarely, on about 5 of 20,000 sections. High levels of inoculum (at least five million conidia/ml) were necessary to get reasonable levels of infection.

Ivory (1967, 1972), working in East Africa, studied infection of P. radiata seedlings by D. pini var. keniensis, again after artificial inoculation with sprayed conidial suspensions. Occasionally the tip of a germ tube was coiled or swollen into a structure he called a vesicle at the edge of or in the stomatal antechamber. Entry into stomata had occurred in two days, and the swollen structures had formed by 5-10 days after inoculation, but the exact time wasn't determined. He did not confirm Gadgil's observations of the large mycelial masses over and in the stomata. These undoubtedly were the result of the particular circumstances of inoculation and incubation used by Gadgil.

Muir (1974) worked with P. radiata seedlings in growth chambers and also made observations on needles from naturally inoculated P. radiata and P. muricata in the field. Again, extensive growth of mycelium was observed on needles of field collections. His SEM observations of naturally inoculated needles showed that spores usually produced one germ tube which grew to the nearest stoma. Only a few instances of growth in the antechamber were observed, generally as a simple germ tube that grew down toward the guard cells. Unfortunately his SEM observations did not reveal details of germ tube behavior in the stomatal antechamber. On cellulose acetate casts a few flat or rounded structures were seen just above the guard cells, but these weren't illustrated. He also described the presence of lobed, flat, reticulate structures in antechambers of needles of both field trees and inoculated seedlings, but details could not be made out with this technique. Apparently, these were not considered the same as Ivory's vesicle-like structures in the antechambers.

Our observations of infected P. nigra needles by SEM revealed details of development after entry of the germ tube into the stomatal antechamber. Most of the cytoplasm concentrated toward the tip and the spore and the remainder of the germ tube collapsed. Within the antechamber the germ tube became delimited from the collapsed portion by a septum. Often several septae developed, the hypha expanded in diameter, became swollen at the tip, and later forked. As development continued a typical 4-footed or quadrupedal structure formed above the guard cells, which was presumably an appressorium. This structure becomes melanized and thick-walled as it develops. As the season progresses it appears that growth of such structures can continue so that they may develop into star-shaped or many-lobed complex and irregular structures.

Red pine (P. resinosa) trees immediately adjacent to the infected P. nigra plantation were not infected and SEM examinations of these also were made to determine the behavior of D. pini on red pine. On all needles examined, stomata were completely occluded with wax and germ tubes could not enter. Germ tubes grew only over the surface of the wax plug, the tips often becoming slightly expanded or contorted, appearing almost as abortive attempts at appressorium formation in response to a stimulus from the stomata.

In further consideration of the possibility of a stomatal stimulus directing germ tube growth to stomata and appressorium development in the antechamber, we prepared plastic needle replicates from silicone rubber molds of Austrian pine needles and placed these among the branches of the Austrian pines for spore deposit and germination. Germination of spores was similar to that on real needles. Germ tube growth was at random, however, and there was no evidence of directed growth toward the stomatal apertures. When germ tubes entered antechambers, growth continued as on the surface, often in a meandering manner and there was no swelling or other differentiation in to appressoria.

On needles of P. radiata from New Zealand, sent to us by Gadgil, germ tube entry and development in the antechamber was essentially similar to that on P. nigra in Wisconsin. Appressoria seemed more irregular in shape and were not always present. Certainly, however, the massive growth over and in stomata originally seen by Gadgil on his artificially-inoculated material was not present.

#### Development in Substomatal Chamber and Mesophyll

Both Gadgil and Ivory gave somewhat similar accounts of the behavior of the fungus in needle tissue. Penetration between the guard cells was by a narrow hyphal strand. The fungus branched in the substomatal chamber, grew in the mesophyll, and was largely intercellular, but Gadgil reported it also as intracellular, probably after the cells were killed. Cells were killed in advance of the hyphae by a toxic material named dothistromin by Bassett et al. (1970).

In epidermal strips of needles of both P. radiata and P. muricata, Muir (1974) observed substomatal vesicles often seen as small sausage-shaped structures. In P. muricata a prominent spherical cell of up to 8  $\mu\text{m}$  in diameter occurred just below the guard cell junction. He speculated that these structures might provide a dormant period for the fungus during dry weather.

Collections of needles from P. nigra in Wisconsin were embedded in Spurr's resin and sectioned either transversely or longitudinally. A few needle segments had been first examined by SEM and then were embedded and sectioned for examination by light microscopy. From one lobe of the appressorium a fine infection strand penetrated between the guard cells and then formed a vesicle of tear-drop to spherical shape immediately beneath the guard cells in the substomatal chamber, varying in size but up to about 10  $\mu\text{m}$  in diameter. The vesicle then produced a single infection hypha which grew into the mesophyll. Occasionally, a secondary vesicle budded off the original vesicle. As development continued other infection hyphae developed and eventually the vesicle lost its integrity. Often the substomatal chamber became filled with a tortuous mass of hyphae. In other infections the substomatal chamber was crossed with only one or two strands of hyphae from the transformed vesicle. Mycelium extended into the mesophyll, but often for little more than about 50 to 150  $\mu\text{m}$  beyond the penetration point. First evidence of the toxic action of dothistromin could be seen by a yellowish discoloration of the cells immediately adjacent to the substomatal chamber when a vesicle had formed, but before the development of infection hyphae. Later, mesophyll discoloration and disruption could be seen for as much as 200 to 600  $\mu\text{m}$  beyond the extent of hyphal limits in individual lesions.

In the New Zealand P. radiata needles similar vesicles were formed. Typically, however, the infection hyphae developed laterally, immediately beneath the vesicle and guard cells to form a structure somewhat resembling an inverted "T".

It seems likely that if Muir's material had been examined in serial sections his spherical cells and sausage-shaped vesicles would have closely resembled the vesicles we saw, particularly those observed in the P. radiata material.

Fruiting stromata began to develop in the fall in P. nigra in Wisconsin, but did not complete maturation and begin spore production until the following spring. The stroma began to form between cells of the hypodermis, or between the hypodermis and epidermis, through which it finally erupted. Even at this stage mycelium was sparse in the mesophyll and the stroma formed close to the point of initial entry into the needle by the fungus.

### Conclusions

The phenomenon of directed germ tube growth toward stomata does occur by this fungus in nature, but this seems to be on an individual stoma basis, or at least it may not occur uniformly for all stomata at the same time.

The development of an appressorium or appressorium-like structure in the stomatal antechamber after entry of a single germ tube is a relatively consistent feature, although apparently penetration between the guard cells may occur without it. The extent of appressorium development is variable, from a relatively simple, typically quadrupedal structure that may almost fill the antechamber. Factors influencing appressorium development and the exact role of the structure in the infection process are unknown.

Also, the formation of a substomatal vesicle after penetration between the guard cells by a fine infection strand seems to be a regular feature of development by the fungus. Infection hyphae, few or many, develop from the vesicle which eventually loses its original shape after budding off a number of hyphae.

Mycelial development in the needle tissue is very restricted in extent. Cells are killed considerably in advance of the hyphae by diffusion of a toxic material from the mycelium.

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THE INFLUENCE OF WETWOOD ON DECAY  
DEVELOPMENT IN BLACK COTTONWOOD

B. J. van der Kamp

Good Morning. Before I start with the last paper of our panel, I want to give credit where due. Most of the work I am going to report on is taken from the Ph.D. thesis of Dr. Atul Gokhale. Atul is now a social worker in Vancouver, another victim of restraints and cut-backs.

Black cottonwood (Populus trichocarpa Torrey and Gray) in our area invariably exhibits a column of dark stained wood in the place where one would normally expect heartwood. This wood has a higher moisture content than inner sapwood (160 vs 125%), a higher pH (7.8 vs. 6.3), apparently low oxygen levels, and a very abundant microbial population. In other words, in black cottonwood, heartwood is replaced by wetwood. Wetwood is of universal occurrence in older black cottonwood in our area. Examination of large piles of logs at a local papermill did not reveal a single log without wetwood. We dug up seedlings and saplings of various ages to determine at which age wetwood first appears. The results are shown in Table 1.

It has traditionally been supposed that wetwood is a deleterious condition damaging to the tree. If this is true, it is difficult to reconcile with universal occurrence of wetwood. From the start we hypothesized that wetwood has a beneficial function, namely that of protection against decay. We established early on that at times the O<sub>2</sub> content of the wetwood could be very low. The main thrust of the study therefore was to determine whether the oxygen content of the wetwood was sufficiently low to prevent or reduce decay. Direct comparison between trees with and without wetwood proved impossible; the latter could not be found. As a result we were reduced to

- (1) Determining the gas composition of the pneumatic system of black cottonwood wetwood throughout the year, and
- (2) Measuring the rate of decay of black cottonwood blocks in vitro under similar conditions.

METHODS

Unwounded black cottonwood and red alder (Alnus rubra Bong.) trees 21 to 41 years old, 21 to 54 cm DBH and 11 to 20 m tall, growing in close association on the University Endowment Lands near Vancouver, B.C. were selected as sample trees. Gases from the wetwood and sapwood of cottonwood and the heartwood of red alder were extracted and analysed in the following manner. A brass pipe (o.d. 0.9 cm), fitted

TABLE 1

Frequency of wetwood in non-wounded black cottonwood trees by age.

<u>Age</u>	<u>Number of trees examined</u>	<u>Percentage of trees with wetwood</u>
1	17	0
2	18	0
3	31	0
4	21	0
5	12	0
6	17	0
7	2	0
8	17	5.8
9	20	10
10	13	53.8
11	12	91.6
12	9	100
13	4	100
14	9	100
15-25	11	100
26-35	25	100
36+	5	100

with a shut-off valve was inserted to the appropriate depth through an increment borer hole. Gases were extracted using a tubing pump. The suction developed and the flow rate were recorded. A Beckman field lab analyser was used to measure  $O_2$  (lowest level of detection 0.05%) while  $CO_2$  and  $CH_4$  concentrations were determined by gas chromatography. The field apparatus consisted of the following items listed in order according to the direction of gas flow: brass pipe in tree, vacuum gauge, tubing pump, gas collecting tube (for  $CO_2$  and  $CH_4$  analysis) with bypass,  $O_2$  sensor, liquid trap, flow meter, vent to atmosphere. All connections were made with 3 mm i.d. vinyl tubing. The wetwood of 6 trees was sampled at bi-monthly intervals throughout 1974. In addition, the wetwood of a further 16 trees was sampled for short intervals, mostly in connection with separate experiments on tree wounding. Gases were extracted at bi-monthly intervals from the heartwood of two red alder trees and analysed in a similar manner.

Extraction of sapwood gases presented a special difficulty. Gas flow into increment borer holes in the sapwood of black cottonwood was generally blocked 4 weeks after they were drilled. No significant volume of gas could be extracted from such holes at the maximum suction developed by our tubing pump (66 kPa). Sapwood gas samples were therefore collected throughout the year from 9 trees, each tree being sampled a maximum of three times. For fear that a hole drilled deep into the sapwood might affect the gas composition of the wetwood, sapwood gases were collected from different trees than wetwood gases.

A modification of a soil-block test (ASTM 1971) was used to determine the extent of decay of black cottonwood under near-anaerobic and aerobic conditions. Polyporus delectans Peck and Ganoderma applanatum (Pers.) Pat., both white rots, were used as test fungi. The former is the most common decay fungus of living black cottonwood in British Columbia, Canada, while the latter is common but virtually restricted to dead cottonwood.

Sapwood and wetwood blocks (2 cm cubes) from 4 black cottonwood trees were cut and sterilized by either gamma-radiation at  $2.5 \times 10^6$  rads (complete sterilization) or by flame sterilization (surface sterilization). The moisture content of each test-block was assumed to be equal to that of an end matched adjacent block. The test was made in 16 oz bottles which contained approximately 80 g of soil, with 27.2% moisture-holding ability by dry weight, and a black cottonwood sapwood feeder strip (5 x 5 x .5cm). After inoculation of the bottles with the required fungi and once the fungi were well established on the feeder strips, 4 test blocks, 1 from each of 4 trees, were placed in each bottle. No further attempt was made to distinguish between the wood of these 4 trees. A disposable anaerobic system (Gas Pak - BBL) was used to create near-anaerobic conditions ( $O_2$  concentrations 0.08%, range 0.06 to 0.10%). The bottles were incubated in the dark at 27° C and 70% relative humidity.

The experiment was done in two parts. In the first part, half the bottles were subjected to near-anaerobic conditions while the other half served

at atmospheric controls (aerobic). After 10 weeks, 2 blocks were removed from each bottle and their weight loss determined (Part 1). In the second part, the same bottles with the 2 remaining blocks were exposed to the environment opposite to the previous one, i.e. near-anaerobic to aerobic and aerobic to near-anaerobic for a further 10 weeks before weight loss assessment (Part 2). At the end of the first 10 weeks the gas composition of 6 of the anaerobic systems was determined. Each of the 32 treatments consisted of 12 replicates (blocks).

## RESULTS

Table 2 gives the composition of gases from the trunk wetwood of unwounded black cottonwood trees. The column headed "Percentage of trees with 0.10% O<sub>2</sub>" was included to show the proportion of trees in which the O<sub>2</sub> level was equal to or less than that used in subsequent decay tests. The O<sub>2</sub> level dropped below 0.10% for a continuous period of 5 to 19 weeks (average 9 weeks), and below 1.05% for 13 to 31 weeks (average 19 weeks) in the 6 trees under regular observation. The highest O<sub>2</sub> level recorded in the wetwood of any tree was 6.2% on February 18th. The wetwood gases of many trees contained no detectable O<sub>2</sub> during May, June and July.

The highest CO<sub>2</sub> concentration recorded was 13.3% on October 15, and the lowest 4.0% on November 22. Some trees had consistently higher CO<sub>2</sub> levels than others.

The variation in the average concentration of CH<sub>4</sub> is largely due to the fact that 3 trees with CH<sub>4</sub> concentrations of about 30, 20 and 15% respectively were sampled at irregular intervals from May to August. CH<sub>4</sub> was detected in three of the six regularly sampled trees. The highest level recorded in these trees was 2.5%. A consistent annual pattern of CH<sub>4</sub> concentration was not evident; the concentration of CH<sub>4</sub> in a given tree changed very little over the year. The highest CH<sub>4</sub> level ever recorded was 34% on May 8. The pressure of the wetwood pneumatic system was always equal to that of the atmosphere. The lowest O<sub>2</sub> level ever recorded in black cottonwood sapwood was 11.4%, the highest 18.8%; CO<sub>2</sub> concentration varied between 0.1 to 0.6% through the year. In the heartwood of adjacent red alder, O<sub>2</sub> ranged from 15.9 to 19.4% and CO<sub>2</sub> from .35 to .88%.

The percent weight loss of black cottonwood sapwood and wetwood blocks under aerobic and near-anaerobic conditions is recorded in Table 3. The composition of gases in the anaerobic systems at the end of 10 weeks was: O<sub>2</sub>, 0.08% (range 0.06 to 0.10%); CO<sub>2</sub>, 6.0% (range 4.5 to 7.0%). The remainder was presumed to be nitrogen with only traces of hydrogen remaining at this time.

## DISCUSSION

### Gas Composition in Tree Trunks

The concentration of  $O_2$  in black cottonwood wetwood was consistently much lower than that in the sapwood or in the heartwood of adjacent red alder. Bacteria and other micro-organisms may play some role in developing and maintaining the low  $O_2$  levels. Part of the reason that  $O_2$  is much lower in summer than in winter may be that these organisms are more active at the higher summer temperatures. It is noteworthy, however, that the highest air temperatures were recorded during the last half of July and August. At this time the  $O_2$  levels were well above the minimum achieved in June.

$CO_2$  levels found in the wetwood were always much higher than those found in the sapwood of black cottonwood or than in the heartwood of red alder. A distinct seasonal trend was evident; the concentration in summer was generally higher than that found in winter.

The marked distinction between sapwood and wetwood gases is noteworthy. The surface area to volume ratio of the wetwood column was as high as  $0.25 \text{ cm}^2 \text{ per cm}^3$ . This suggests that in order to maintain the low  $O_2$  levels the rate of diffusion from the sapwood to the wetwood must be rather low, and the oxygen demand of the wetwood high. There were no visible barriers to the radial movement of gases between sapwood and wetwood or within the wetwood. The boundary between sapwood and wetwood was only roughly parallel to the cambium, and commonly crossed two or three annual growth rings.

The low  $O_2$  and high  $CO_2$  levels in the wetwood of black cottonwood appear to be peculiar to this tree species. Red alder, growing in close association with our cottonwood sample trees exhibited  $O_2$  levels in the heartwood close to those found in the atmosphere while  $CO_2$  was always below 1%.

### Decay Development Under Near-anaerobic Conditions

There was no statistically significant weight loss in any of the treatments exposed to near-anaerobic conditions for 10 weeks, while weight loss of control aerobic treatments varied from 29.5 to 47.6% (Table 2, part 1). Therefore, it is most probable that under field conditions during summer, the concentration of  $O_2$  found in the wetwood of many unwounded trees is too low to allow any development of decay, at least by our test fungi. It should be pointed out, however, that in our experiments the fungi survived the 10 week near-anaerobic period and presumably the same might happen in the field.

The level of decay development on wood blocks under near-anaerobic conditions was less than 1% of that of aerobic controls and not significantly different from zero. On agar the linear growth rate

TABLE 2

Composition of gases from the trunk wetwood of unwounded black cottonwood trees.

Sampling Period (1974)	Average Concentration (%)			Number of trees sampled	Percentage of trees with:		
	O <sub>2</sub>	CO <sub>2</sub>	CH <sub>4</sub>		<1.05% O <sub>2</sub>	<0.10% O <sub>2</sub>	detectable CH <sub>4</sub>
Jan. 1-31	2.22	5.40	0.14	13	15	0	15
Feb. 1-15	2.57	5.30	0.17	7	0	0	28
Feb. 16-28	1.78	6.28	0.08	6	50	0	17
Mar. 1-15	1.66	6.97	0.20	6	67	0	33
Mar. 16-31	1.80	6.78	0.17	6	50	17	33
Apr. 1-15	1.11	6.76	0.20	5	60	20	40
Apr. 16-30	0.52	6.65	0.48	6	83	50	50
May 1-15	0.41	7.70	4.09	9	78	67	56
May 16-31	0.23	8.88	2.76	12	100	67	42
June 1-15	0.03	8.44	3.92	11	100	82	73
June 16-30	0.02	8.46	0.74	7	100	86	57
July 1-15	0.20	8.49	0.89	9	100	89	56
July 16-31	0.35	8.59	4.05	9	100	44	67
Aug. 1-15	0.80	8.85	0.25	6	83	0	50
Aug. 16-31	1.16	7.16	3.96	12	33	17	67
Sept. 1-15	1.80	7.93	0.50	6	17	0	50
Sept. 16-30				0			
Oct. 1-15	2.00	7.41	1.15	8	12	0	62
Oct. 16-31	2.49	8.59	0.40	8	0	0	50
Nov. 1-15	2.23	8.10	0.53	6	33	0	50
Nov. 16-30	2.90	6.35	0.42	6	17	0	50
Dec. 1-31	2.56	6.43	0.48	8	38	0	50

of P. delectans and G. applanatum under near-anaerobic conditions was 4 and 12% respectively of that of aerobic controls. It appears therefore that the activity of these decay fungi under near-anaerobic conditions depends on the substrate on which the test is done and perhaps also on the parameters used to measure such activity.

The experimental design of the decay test permitted distinction between the effects of O<sub>2</sub> levels, bacterial presence, pH, and any toxic compounds. If bacteria had a direct influence on the rate of development of decay one would expect significant differences between the weight loss of surface and completely sterilized wetwood blocks under either or both aerobic and near-anaerobic conditions. No such difference was detected. If pH were a factor, one would expect a difference between sapwood and wetwood blocks. Again no such difference was detected. If there were toxic compounds in the wetwood not found in the sapwood, one would expect a lower rate of decay in the wetwood. Again this was not observed. The only factor that appeared to affect decay development was O<sub>2</sub> concentration.

There was no significant additional weight loss during the 10 weeks exposure to near-anaerobic conditions following 10 weeks of aerobic conditions. The maximum weight loss of cottonwood blocks decayed by the two test fungi in other experiments over longer time periods exceeded 70%. This suggests that even if a decay fungus is well established and actively growing in a piece of wood, imposition of anaerobic conditions appreciably inhibits further decay activity. Presumably the same would happen in a tree.

It is noteworthy that the weight loss of blocks exposed to near-anaerobic conditions followed by aerobic conditions was much lower (average 10.9%) than that of blocks exposed to aerobic conditions only (average 41.7%). Perhaps toxic metabolic by-products accumulate under the near-anaerobic conditions and their elimination is required before normal growth resumes. Alternatively, it is possible that during the near-anaerobic period, living mycelium of the fungus is reduced to special resting stages thus substantially reducing the inoculum potential. A similar phenomenon might reduce the activity of fungi in trees during winter months following near-anaerobic summer conditions. Furthermore, decay activity during the winter months would also be limited by low temperatures.

We expected that since P. delectans occurs in living trees, even though commonly associated with scars or branch stubs, while G. applanatum is normally restricted to dead trees, the former might be more tolerant to near-anaerobic conditions. However, neither fungus caused a significant weight loss after 10 weeks under near-anaerobic conditions, therefore indicating no preferential ability of P. delectans to tolerate such conditions. In a sequence of 10 weeks near-anaerobic followed by 10 weeks aerobic, however, the average weight loss caused by

TABLE 3

Percent weight loss of black cottonwood sapwood and wetwood blocks under aerobic and near-anaerobic conditions after 10 and 20 weeks.

Fungus	Wood zone	Sterilizing method	Percent Weight Loss						
			Part I (10 weeks)			Part II (10 weeks + 10 weeks)			
			aerobic	near-anaerobic	aerobic + near-anaerobic	aerobic + near-anaerobic	aerobic + near-anaerobic	aerobic + near-anaerobic	
<i>P. delectans</i>	Sapwood	surface	29.5*	2.9**	-0.1 .4	29.4	4.6	5.0	2.5
		complete	47.6	2.8	0.3 .3	51.6	4.7	7.8	2.1
Wetwood	Wetwood	surface	38.7	3.9	0.8 .5	45.5	3.6	7.7	2.1
		complete	41.8	3.0	-0.5 .8	37.2	5.6	9.2	2.5
<i>G. applanatum</i>	Sapwood	surface	42.5	4.0	0.3 .8	49.3	2.8	14.7	3.5
		complete	43.5	2.9	0.3 .7	43.0	2.7	15.7	3.1
Wetwood	Wetwood	surface	43.2	3.3	0.3 .7	45.9	1.0	16.9	4.3
		complete	46.7	3.1	-0.1 .7	44.8	4.6	10.6	3.0

\* each weight loss is the average of twelve blocks

\*\* standard error of the mean in italics

P. delectans was 7.4% while that of G. applanatum was 14.5% suggesting a more rapid recovery from near-anaerobic conditions by the latter. Presumably the occurrence of these fungi in the field is determined by factors other than those considered here.

Any wound or opening into the wetwood would naturally admit O<sub>2</sub> and negate any decay resistance due to low O<sub>2</sub> levels. However, branch stubs do not function as such openings. Our sample trees had many branch stubs and still were able to maintain near-anaerobic conditions in summer.

Many investigators dealing with wetwood have regarded it as a deleterious phenomenon. As a result of our work we suggest that in black cottonwood the occurrence of wetwood may well be a perfectly natural phenomenon which imparts a considerable degree of decay resistance to the inner wood.

PANEL: BIOLOGICAL CONTROL OF TREE DISEASES

ED F. WICKER, MODERATOR

PANEL INTRODUCTION

Control of plant diseases with biological agents is very appealing from the standpoint of procedures, costs, and the absence of residue problems. Such control is only a segment of natural control which I consider to be the summation of control contributed by all natural agents and factors. These biological agents are a very integral part of the suscept-pathogen's environment and foreign or toxic substances seldom are introduced into the environment when using such control measures.

Biological agents are known to affect fruiting, growth, and survival of many plant pathogens, some of which are highly specialized or even obligate parasites. Also, they are known to ameliorate the effects of disease on the suscept.

Investigations of biological control of plant disease have not received the scope they merit. The role and significance which this control measure should occupy have not been realized (Fedorinchik, 1964). The research needs to thoroughly explore potential avenues for biological control of plant diseases are long standing, such needs immediately become stark reality when we consider the logic and ecological necessity for developing integrated measures for effective management of plant pest problems. This becomes more and more apparent as agricultural production acquires a stable trend of narrow specialization in raising crops.

The use of resistant varieties as the major method for plant disease control cannot keep pace with adaptability of the causative agents because of the latter's greater plasticity and ecologic amplitude and their shorter reproductive cycles. As agricultural production becomes even more highly specialized, biological control methods will assume their proper role as a major factor of integrated control measures for plant diseases (Fedorinchik, 1964).

Only a few biological control agents of tree diseases have been studied in the detail necessary to adequately evaluate their potential usefulness. Plant pathologists and foresters have not been attracted by the biological measures of plant disease control because such measures are often (1) difficult to appraise; (2) slow and erratic in effecting control; (3) less spectacular in achieving control; (4) biologically complex and, therefore, more difficult for man to understand and manipulate; (5) not economically effective unless manipulated by man; and (6) less likely to

be complementary to other control measures in an integrated control program than are cultural, chemical, or genetical control measures. Furthermore, control is relative. Consequently, prior to 1970, most of the knowledge acquired is a result of mycological curiosity and motivation. Thus, the majority of the available information concerns structure of the active agents. Data on function is necessary for evaluation of their potential as biological controls. A detailed understanding of their Mode of Action is imperative to the evaluation of potential biological control agents for controlling plant diseases.

I often have occasion to go back in time and review the proceedings of past Western International Forest Disease Work Conferences. My latest excursion through these annals of wisdom revealed that we conducted a panel discussion on biological controls of forest disease during our 19th conference in 1971. Those of you who may have embarked also on such a journey in quest of intellectual enrichment and trodden the pages of past conference proceedings, will notice some duplication of subject matter covered by the panel in 1971 compared with that to be offered by the present panel. I refuse to become alarmed at this revelation, because I have complete confidence in my ability to evaluate and select highly-competent panel members for this discussion topic. I was not involved with the panel in 1971, and therefore, I will terminate any further innuendo and confine my comments to introduction of the present panel.

Speaker #1: Dr. John Staley

Subject: Biological Control of Needle Diseases.

Speaker #2: Dr. Neil Martin

Subject: Biological Control of Root Rots.

Speaker #3: Dr. Ralph Williams

Subject: Biological Controls for Nursery Diseases.

Speaker #4: Dr. Ed F. Wicker

Subject: Biological Control of Conifer Rusts.

THE POSSIBILITIES AND POTENTIAL OF BIOLOGICAL  
CONTROLS FOR FOLIAGE DISEASES OF CONIFERS

John M. Staley

There is strong evidence that under greenhouse and experimental conditions biological control by infestation with saprophytic bacteria prior to foliar pathogen inoculation can decrease remarkably the development of certain diseases (4,6). There is no evidence provided by field experiments that a practical biological control with bacterial epiphytes can be obtained for foliage diseases under less carefully controlled natural environments (5). In fact, the evidence suggests that, under naturally fluctuating conditions, the few biological control agents known to work under experimental conditions are soon eliminated by drying or other adversities. However, if fire can be conceived as a biological control agent as it has been in some author's treatments (3,8), then we can point to the case of brown spot control in longleaf pine by fire as a successful application of a silvicultural treatment designed to control a needle pathogen through a combination of eradication and environmental modification.

The idea that epiphytic microorganisms might play a part in diseases incited by foliar fungi is not new. Leben (4) in his 1965 review of these relationships traces the idea back almost 70 years. Many treatments of needle disease pathogens note in detail the secondary fungi such as Hemiphacidium planum or Hendersonia acicola that commonly fruit on attacked foliage. These accounts usually mention that such secondary fungi may be instrumental in the ultimate natural diminution of damage seen in connection with needle cast epiphytotics. To my knowledge, there has been no experimental effort to grow these secondary parasites in culture and apply their spores artificially, in attempts to control needle-casts.

There are a very few experimental studies relating to biological control of foliage diseases of forest trees and with the exception of those involving fire, only two of these relate to conifer foliage diseases. It may be helpful to bring out a few details of these studies to illustrate where we stand as stated in my opening summary.

In 1966, J.E. Bier (1) presented his results of pre-treatment of Populus trichocarpa foliage with non-sterile leaf washings prior to inoculation with Melampsora occidentalis. On 267 branches, results were equivocal on older foliage, but on the youngest three leaves differences significant at the 0.1% level were observed. Reductions in infection and pustule formation on these youngest leaves were on the order of 25% and 90%. Such results show unequivocally a beneficial effect of the treatment, but still leave much to be desired as a practical means of control.

In 1969, R.P. McBride (6) reported that, in greenhouse trials, reduced infection of Melampsora medusae resulted from treatment of Douglas-fir seedlings with pre-inoculation atomizations of bacteria containing broth and, to a lesser degree, bacterial suspensions and cell free filtrates of nutrient broth cultures. Over 98% reduction in numbers of seedlings diseased was achieved. Numbers of bacteria on the foliage seemed to be correlated with reductions in infection, and a mixture of bacteria. The reduction in seedling infection thus seemed the consequence of bacterial action, whether this was nutrient competition or antibiosis, or both.

In 1971 McBride (7) presented a similar study of larch seedlings showing that bacterial suspensions would cause significant reduction in infection by Meria laricis. These amounted to a 30 to 60% reduction in infection. The inoculations were conducted in the greenhouse to seedlings held in polyethylene bags.

The work relating control of brown spot of longleaf to elimination of the inoculum source and modification of the environment by removal of surrounding grass and weeds has already been cited as a practical means of silvicultural manipulation to control a damaging foliage disease. By this method, the percent of diseased foliage may be reduced as much as 40% to 2% and the reduction maintained to a lesser degree up to four seasons. Controlled burning at three-year intervals until the desired number of seedlings are over 18 inches in height is recommended for successful control (8).

One can go yet a bit further afield to note Burchill and Cook's (2) report that 5% urea sprays promote chemical and microbial changes in apple leaves that prevent the development of Venturia inequalis on overwintered fallen leaves.

With the exception of brown spot control by fire, each of these reports of modified biological activity in leaves or on their surfaces, involves the application of some material to the leaf to effect the disease and/or inoculum reduction. From the standpoint of forestry, there are limited circumstances under which such disease control applications would be economically justified. Most foliar disease damage under forest conditions would probably not begin to justify even a single such direct application. However, if such treatments were both highly effective and lasting, they might find application in Christmas tree culture or in forest nurseries.

It seems, therefore, in biological control of coniferous foliage diseases with epiphytic microorganisms, we have at present only some encouraging evidence that a potential control means exists, and that there is a blank spot in our understanding of it. Should microbial epiphytes play a significant role in controlling coniferous foliage

disease, it seems unlikely that they could economically be employed to do this by means of direct application, except in those special cases where the value of the crop is high, and its term of production short.

We should, however, try to fill this gap in our knowledge both to perfect such possible direct applications and perhaps more to the point, to investigate the possibility that leaf epiphyte populations might be modified to our benefit through silvicultural manipulation. Since such studies have neither been done nor ever planned, I take this as an opportunity to discuss how they might be approached.

With the exception of the Venturia-urea relationship, the favorable observations to date concern relationships between natural processes that have been brought together within an unnaturally short time span by artificial inoculation. Conifer foliage diseases in nature appear to be quite the opposite. They are the result of extended and repeated inoculation processes, and are further characterized by prolonged and variable latent periods. Epiphyte antagonist population would thus have to be maintained at an effectively high level over a considerably longer period than that involving a single experimental inoculation. In contrast to the single pre-inoculation treatment favored in experiments to date with bacterial antagonists, the secondary parasites of conifer pathogens would appear to require an initial buildup of infected foliage which they might then penetrate and fruit upon. Thus, conifer foliage diseases don't fit well the experimental models that provide us with our glimmers of possible success in using epiphytic microorganisms to achieve biological disease control.

Foliage diseases of Christmas trees and nursery seedlings such as that caused by Lophodermium seditiosum (recently segregated from L. pinastri) have a fairly short latent period. They are sufficiently pathogenic to largely overcome host resistance and, therefore, provide a reliable amount of material for replicated studies. Moreover, direct applications of control substances appear necessary to successfully grow disease free individuals, at least in some environments. In the forest, Lophodermella morbida is an example of a foliage disease that has a short latent period, is strongly pathogenic, and is predictable in occurrence. Another feature favoring its use in initial studies is that it also is parasitized by Hendersonia acicola, a fungus, the effect of which would be interesting to study after the foliage disease had taken hold. Many other diseases such as Davisomycella, Virgella, and Elytroderma for example, seem too unpredictable in their response to weather or have too prolonged latent periods to be attractive for study. Whatever disease relationships is chosen for study, strong pathogenicity and predictability, over a short time span of observation, seem requisite.

In each of the suitable diseases for study, individual trees can be observed that are possessed of resistance. Perhaps these degrees of resistance are related to the epiphytic microorganisms and the specific ability of such trees to support microbial growth through leakage of nutrients from the leaf. We could perhaps take a leaf from the book used by soil microbiologists and begin our search for antagonists on such trees. We might compare the microflora of resistant trees with that of susceptible trees. Judging from work such as Leben's, such efforts will probably be laborious. Leben examined and tested 230 isolates from cucumber before finding one that had antagonistic potential against cucumber anthracnose. For forestry purposes, it might be well to start the study testing Hendersonia acicola effects against Lophodermella morbida. Hendersonia has been grown in culture. It would be a matter of developing techniques to allow abundant sporulation in culture, followed by the successful inoculation of the spores on attacked foliage. The technique would have to be effective enough to overcome the variability introduced by the natural occurrence of Hendersonia on untreated trees. It is possible that treatment such as a urea spray would exert an effect on the secondary parasite that would be worthy of study. On the other hand, such secondary parasites would be next to useless for the Christmas tree grower, and probably also to the nurseryman.

McBride has shown for larch what may well be true for most conifers: that bacteria and yeasts are the initial colonizers of new foliage and that non-pathogenic fungi occur only as spores on young leaves, hyphal development being restricted until the leaf has reached maturity. Presumably it is among these bacteria and yeasts that we are likely to find effective antagonists capable of acting in what appears to be the primary inoculation court for foliar disease fungi, that is, the immature newly-formed leaf. Isolation of these organisms, at least in the case of bacteria, requires the use of low nutrient media suited to their growth. Presumably a candidate antagonist would be one of the more common isolates, innately suited to flourish on the surface of foliage and well adapted in its resistance to adverse fluctuation of the environment. It should, in other words, be a fairly common organism found associated with healthy plants growing in an environment favorable to disease development.

Testing of such an organism might well proceed by using the branch dip technique that has been so satisfactory in screening fungicides. Apparently suspensions of organisms grown in their nutrient media without further preparation have been satisfactory in screening antagonists under experimental conditions. Judging from the success of previous trials, care should be taken to assure that approximately 500,000 propagules per square centimeter of foliage are used.

Assuming that antagonism and a degree of disease control can be successfully demonstrated, it then seems incumbent on the pathologist to demonstrate how the microbiological epiphyte antagonist can best

be nurtured under forest conditions by silvicultural practice. One assumes that, if epiphytic antagonists are, in fact, related to disease resistant individuals, the silvicultural practice most suited would be to plant the superior disease resistant offspring of selected superior plants. I think, however, that the basis of disease resistance in superior trees is not likely to be merely their epiphytic complement of organisms and that the pathologist might better fall back on observing those cultural situations under which the desired microbes flourish and foliage disease is absent. The task would then be to experiment by practicing imitation of nature to see if the desired disease-antagonistic microbes can be promoted through silvicultural treatments.

Fire has not been explored in the western United States as a tool to control foliage diseases. It might be effective under some circumstances if high enough temperatures could be achieved to kill the lower infected foliage without damaging the lower holes. This hazard of tree mortality would have to be demonstrably low for fire to be useful in controlling a disease which itself kills infrequently. Two past occurrences of foliage disease, that of Lophodermella cerina in Arizona and Lophodermella morbida in Oregon have been over extensive enough areas to have allowed experimental tests of fire to be used. The more spotty and unpredictable needlecast outbreaks do not lend themselves to experimental tests of fire's effectiveness.

Leben (4) in 1965, described microbial antagonism as a fourth dimension in the study of foliar disease. We are really no closer in 1978 to a knowledge of how important this dimension is to the forest pathologist than he was then.

At present, in the western United States, we would seem to have to fall back on the tried and true preventative measure of choosing the appropriately resistant host species for a given site. All other biological control measures or silvicultural manipulation to achieve biological control are at this point only in the speculative and experimental stage.

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## BIOLOGICAL CONTROL OF ROOT INHABITING FUNGUS PATHOGENS

Neil E. Martin

### Philosophy of Why Biological Control

The protection of agricultural crops, forests, and man and other animals from plagues and damage by various kinds of pests remains a never-ending problem. Our entire life style is a continuous struggle to provide an even better life style. For example, as a person and as a nation we spend almost all of our money and energy on protecting life and the essentials to life, only to see our successes give way to new problems (4). Because protection of food and fiber is vital to ourselves and future generations, we must give priority to all potentially useful techniques for the control or management of pests. We must integrate them so their effects are compounded beyond that achieved separately.

One such approach to the management of soil borne organisms appears to be biological control. Biological control catches one's attention because the word "biological" elicits a softer, less violent reaction in the public arena than does the announcement that chemical controls are being considered. Biological control also numbs our sensitivity to energy crises because something biological is seemingly synonymous with self-perpetuating whereas "chemical" conjures up images of boiling vats, storage tanks, menacing liquids and powders, and a threat to the purity of the food and water we eat and the air we breath. Besides, we all know that as ruthless as chemicals are they cannot do the complete job (6). Their numbers are limited by our imagination and therefore cannot combat all situations. They do not enjoy an increase in numbers through multiplication or replication and cannot therefore flex with the target population.

### Mechanisms Evident in Biological Control Agents

The basis for biological control is found in the concept of a balanced ecosystem (2). When an organism can influence in a detrimental manner the population level of another then it is displaying a characteristic of biological control that may be exploited. The mechanisms through which one organism influences another are seldom understood though they seem to be limited to 1) induced resistance in the host plant, 2) hyperparasitism, and 3) antagonism.

Induced resistance requires that when pathogen and host interact, the response makes the host more resistant to a second pathogen (1,2). These phenomenon are known in literature as acquired resistance, cross protection, interference, and simply interactions. Treatment of these subjects is beyond the scope of this presentation regarding forest tree root problems and yet may someday prove to be the most powerful of all biocontrol approaches.

Hyperparasitism, the parasitizing of one parasite by another, is frequent in nature but its significance under field conditions is difficult to assess. The successful management of an undesirable fungus pathogen by another fungus parasite is not known. Attacks by virus and bacteria detrimental to fungus populations are documented and show attributes that may be of value when combined with a hyperparasitic fungus.

The most common mechanism of influence of one organism upon another is antagonism. The mechanisms likely to be responsible are competition for substrate, occupancy of an ecological niche, production of metabolic products, or antibiotics. Evidence for these mechanisms was most often obtained in greenhouse studies or in petri dishes, but the inferences are strong enough to encourage field application of cultural practices that encourage antagonistic organisms.

### Source of Antagonists

Regardless of antagonistic mechanisms, there are no procedures that predict which organisms will function as antagonists (1,2). Antagonists are where you find them. Pest problems are in general man caused. Man has been responsible for introducing plants and animals into ecosystems lacking natural enemies to these exotics (1,2,5). The result has been a rapid unchecked spread exceeding a tolerable threshold. Antagonists are likely to be found in the original area in which the exotic was developed. It is in such areas that a balance existed due to environment, host, and antagonist interactions; however, the antagonistic attribute may not be expressed in the new environment.

### Use of Biological Control

To date, we have not encountered a problem in the Western States with exotic root inhabiting organisms in forest trees, but the warning is recorded in pathology history. Future generations demand more fiber, and to meet those needs we are responding with huge container stock programs, tremendous energy inputs into site amelioration, and human selection of genetic combinations. In all cases, the risk increases as we deviate farther and farther from an indigenous balanced ecosystem.

Some of our problem organisms require rather sound plant parts in which to survive until another generation of hosts is available. Rapid decay of plant remains would seem then to be a viable hypothesis. Risbeth has reported limited success in encouraging decay by injecting stumps with nutritive as well as fungistatic chemicals (2). To ameliorate the forest soil with urea, sludge, or compounds to influence pH is at best temporary because of the enormous buffering capacity of the soil (3), and yet can be effective if timed correctly according to the biology of the organisms involved. Escape and crop rotation are still the most reliable cultural practices that we have. Preventing damage to roots, not exceeding the carrying capacity of the site, and maintaining a vigorous stand all contribute to escape. Shortened rotations and species mixture in effect achieve the principal of crop rotation.

Biological control is centuries old in the forest whether it be above or below the soil surface. Man is just beginning to learn of its potential in helping to reduce losses to all kinds of pestilence. Our employment of biological control agents will increase and improve as we learn more about the biology of forest ecosystems.

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## BIOCONTROLS FOR DISEASES OF NURSERY CROPS

Ralph E. Williams

Annual loss directly or indirectly related to disease agents in bare root seedbeds of the Coeur d'Alene Nursery has been estimated at nearly \$400,000. Losses in greenhouses alone may reach 10 percent of a 2.5 to 3.0 M base. Production capacity (ca 24 M) has been reached, yet future Regional needs will be nearly a third greater than capacity by the early eighties. Alternatives to meet these needs are to put more ground into production or to maximize production. Perhaps improved utilization would be the most economical from both a quantitative and qualitative standpoint.

Various means of improving utilization may be through the use of biocides, sanitation measures, or biological control. Although only biological control will be discussed, most agree that biological control should be regarded as only one facet of the overall control program, rather than as a method to be used alone.

The concept for biocontrol followed in this paper is that of Baker and Cook (1974):

"Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists."

Based on this concept, there are two general approaches to achieving biological control: 1. Introduction into the system of antagonists--(microorganisms which affect pathogens via: (a) antibiosis and lysis; (b) competition; or (c) parasitism and predation; or 2. manipulation of the environment to enhance resident antagonists and/or host vigor. In either case, manipulations to achieve success may ultimately be reduced to a chemical or physical level as it directly or indirectly affects pathogen, host, or antagonist.

It seems clear that for biological control to be effective, it is necessary to create an environment which will: (1) favor vigorous growth of the host which may in turn directly and/or indirectly reduce disease severity; (2) directly suppress pathogen population or activity; and (3) favor antagonists of the pathogen. Even though much information concerning the effects of environmental alternation on host/pathogen/antagonist systems has been published, for many--because of the complexity of soil.

systems whereby many ordered responses are illicited--the exact mechanism of action may not be understood or even identified and may be the reason for the many seemingly contradictory findings and exceptions to general trends.

It is not worthwhile discussing known chemical and physical details of every host/pathogen/antagonist system remotely related to conifer nursery diseases. Only major diseases encountered in Inland Northwest nurseries will be discussed. Suggestions for reducing damage by using biological measures are based on (1) host/pathogen/antagonist information about major nursery diseases, (2) general trends identified in available pathogen/antagonist information from field crops research; and (3) nursery management practices.

Damage caused by Botrytis cinerea in greenhouses, shadehouses, and seedbeds is the major foliar problem, while damage caused by possible Lophodermium sp. may be increasing.

In greenhouses and outdoor storage facilities for greenhouse seedlings, Botrytis has caused considerable damage to western larch and occasional damage to pines during wet periods in late winter, early spring, and fall months. In seedbeds, damage is observed to develop in the early spring months only in dense areas of 2-0 western larch seedlings.

Botrytis cinerea is a relatively nonspecialized facultative parasite (Baker and Cook 1974) requiring high spore densities, senescent or dead tissues in contact with living host tissue, free moisture, and cool temperatures for infection to take place (Baker et al. 1954). Although sanitation and/or biocide treatment may reduce populations of this rather ubiquitous agent, if suitable host substrate uncolonized by other microorganisms is present and if proper environmental conditions exist, infection will likely occur. It seems logical to assume that biological control via environmental manipulation and/or introduction of antagonists may be helpful in reducing damage.

In the Northern Region, manipulation of the greenhouse environment during winter months or shadehouse during late spring or fall to suppress the pathogen's competitiveness while maintaining proper conditions for seedlings is difficult. However, reduced watering, increased air movement, elevated temperatures, and wide spacing of seedlings are alternatives to consider.

The seedbed environment is less easily manipulated than that in the greenhouse. In these situations, propagation of western larch in areas with good air drainage, lower sowing rates to achieve wider spacing, or cultural methods to suppress growth during the 1-0 year may be beneficial.

It appears as though foliar application of antagonists competitive for free carbon- and nitrogen-containing materials may be effective in reducing damage if applied prior to colonization by Botrytis (Newhook 1957, 1951; Blakeman 1972), and if high humidities and moderate temperatures prevail. Perhaps a shotgun approach, applying soil suspensions to foliage, may also be effective. Application of antagonists should be accomplished during the fall or end of the growing season in greenhouses when needles become senescent and when temperatures and relative humidity are conducive to their growth.

Although not positively identified, a pine needle disease problem, perhaps caused by species of Lophodermium, may be increasing in nursery seedbeds. If a needle pathogen of this sort is present, obvious controls are to remove the infection source--surrounding trees and pine needles applied to reduce frost heaving. However, since most nurseries in the Northern Region are in forested areas, this alternative may not be practical.

Biological control via environment manipulation may not be of much use because of current management practices. An exception to this may be related to aluminum levels in soils (Iyer et al. 1971). For the most part, perhaps the only hope for biological control would be through the inoculation of competitive-type antagonists onto seedling foliage.

Major nursery disease problems are soil related, involving primarily Fusarium spp. (Fusarium oxysporum and possibly Fusarium roseum). Damage occurs in both greenhouse and seedbeds and, judging from seed isolations, disease occurrence, and soil testing, inoculum is both seedborne and soil-borne. Therefore, control measures which will reduce effectiveness of either or both types of inoculum, depending on the situation, must be employed.

Seed treatment with biological agents may be effective in reducing germinant infection. Several studies with cereal crops (Merriman et al. 1974; Chang and Kommedahl 1968; Aldrich and Baker 1970) indicate that seed treatment with organisms such as Streptomyces spp., Bacillus subtilis, and Chaetomium sp. has been tested and appears to be effective in reducing crop loss due to infection by Fusarium spp. Seed treatment is essential in greenhouse culture where potting media is not contaminated. It may be effective in reducing losses in seedbeds where both the rooting medium and seed may be contaminated, if activity continues until mycorrhizal development has taken place, at which time a certain amount of long-term disease tolerance is attained (Marx 1972).

Reduction of inoculum potential of soilborne pathogens is normally accomplished directly by the use of biocides. However, where considerations make it undesirable to chemically eliminate these organisms from soils (Iyer et al. 1969; Lu 1968) or biocides do not produce the degree of control desired, biological controls should be considered.

Addition of alien antagonists to soils has generally been ineffective in reducing inoculum potential of soilborne pathogens unless the antagonist is well suited to the system, it is introduced in large quantities, or it is given a competitive advantage by treatments such as fumigation. One notable exception may be the incorporation of mycorrhizal fungi into nursery soils (Marx 1972).

In many nurseries, because fumigation is a standard practice, introduction of antagonists may be effective for at least a short time until the system reverts to a balanced state. In soil systems, only a few organisms have been identified as being antagonistic to Fusarium spp. These include Bacillus subtilis (Mitchell and Alexander 1962), Streptomyces spp. (Bloomberg 1973), perhaps species of Pseudomonas (Kawamoto and Lorbeer 1976), and Trichoderma viride (Wood and Tveitt 1955). Direct introduction has not been tested; however, information concerning environmental manipulation to favor these organisms is available and will be discussed later.

The introduction of mycorrhizal fungi and subsequent mycorrhizal establishment offers not only a protective mechanical barrier to root infection by pathogens, but may also function via antibiosis and stimulation of competitive antagonists (Marx 1972; Zak 1964). Sinclair (1974), however, reports that the mycorrhizal association may not be developed for 7-12 weeks or more. Thus, in light of Bloomberg's work (1973) which shows that much infection of Douglas-fir seedlings by Fusarium oxysporum f. sp. pini occurs early in the life of the germinant, if one were to rely solely upon mycorrhizae for disease reduction, considerable loss could occur. Perhaps inoculation of seed or soil with a mixture of antagonists, as indicated above, to protect the germinant for 7-12 weeks, plus soil inoculation with suitable mycorrhizal fungi, may be effective in reducing disease.

Manipulation of the soil environment to stimulate resident antagonists may be worthwhile since residents are at least partially suited to the system. Indeed, various nursery management practices which are employed primarily to create an environment favorable for tree growth may also be effective in biological disease control. Examples of environmental manipulation as related to biocontrol follow.

Addition of inorganic materials, particularly nitrogen fertilizers, can be important. Nitrate nitrogen tends to suppress soilborne Fusarium spp., while ammonium nitrogen favors them (Huber 1966). It is suggested that the primary mode of action is through changes in pH (Smiley 1975). Soil

reaction adjustment by liming may likewise suppress severity of fusarial pathogens. Low soil pH favors their activity (Garrett 1956; Baker and Cook 1974), while neutral to slightly acid pH suppresses it, but stimulates bacteria and actinomycetes (Alexander 1961), several of which may be highly antagonistic to these pathogens (Bloomberg 1973; Mitchell and Alexander 1962).

Incorporation of cover crops or other organic amendments into soils may effect changes which not only suppress pathogen activity, but more importantly changes which favor antagonists. These changes may benefit specific antagonists as is the case with Bacillus subtilis stimulation with soybean residues (Mitchell and Alexander 1962), but will most often stimulate a variety of competitive types of antagonists as is the case with sawdust incorporation (Lu 1968; Switzer and Nelson 1967). Effectiveness of cover crops in reducing damage depends upon nutritional status of the crop, timing of incorporation, crop maturity, and cropping sequence (Huber et al. 1965; Maloy 1960). In general, mature cover crops with high C:N ratios will reduce severity of fusarial pathogens (Garrett, 1956). Organic amendments which increase moisture holding capacity, pH, and anaerobic respiration, and have high C:N ratios will likewise reduce damage by fusarial pathogens (Baker and Cook 1974; Cook 1976).

Biocide treatments, such as application of various herbicides, may be indirectly effective in disease reduction by affecting nitrogen cycling, which in turn affects populations of soil microorganisms (Huber, 1966; Huber et al. 1969; Baker and Cook 1974). In this case, multi-ordered reactions are responsible for disease reduction. Fumigation and the application of specific fungicides may increase numbers of several organisms which may function as antagonists to the Fusarium spp. (Vaartaja 1967; Bloomberg 1965).

Maintaining soil moisture at 0-1 bar (ca 75 percent field capacity), which is normal for nursery soils (Bloomberg 1973), may not only favor seedling development but may also favor antagonistic bacteria and actinomycetes. High moisture levels may also affect soil aeration which may enhance anaerobic bacterial activity, which in turn may increase ethylene, an agent suppressing activity of several root pathogens (Cook 1976).

Maintaining soil temperature below 23°C., at least during germination periods by shading devices, evaporation cooling, and even perhaps with mulch cover, should reduce severity of Fusarium oxysporum damage to coniferous seedlings (Bloomberg 1973, 1976).

Because of the complex dynamic nature of soils and the interactions between many management practices as shown above, it is easy to see that

the imposition of a simple factor into the system will create multiordered reactions throughout the influence zone. The fact that we are dealing with a very complex system which we may never fully understand should, however, not deter us from using biocontrols. Rather, in the words of Baker and Cook (1974), we should "isolate the phenomenon, make it work, then study it carefully to perfect its accomplishment."

An approach to biological control in nurseries could be to identify areas, either with ground surveys or with remote sensing techniques such as color infrared photography, where problems are significant or nonsignificant. Correlation between problem occurrence and original soil type or management activity maps could be identified. Finally, beneficial practices, either determined from past management or from field crop literature, could then be employed in the appropriate areas.

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## AN EXAMPLE OF BIOCONTROL OF STEM RUSTS OF CONIFERS:

### THE PURPLE MOLD

Ed F. Wicker

### INTRODUCTION

Suppression of native rust fungi throughout the world is due, in part, to their natural enemies which are primarily other fungi. Arthur (1929) lists 37 fungi attacking rusts or living in intimate association in vivo with them. One fungi, Darluca filum (Biv.) Cost., is associated with at least 71 species of rust in Latvia along (Kirulis, 1942). However, of the many fungi known to associate with the rusts, Tuberculina spp. are the most effective at inhibiting their normal growth and development (Tubeuif, 1901, 1930, 1936; Lind, 1913; Weir and Hubert, 1917; Wilson, 1925; Brooks, 1928; Deacon, 1939; Hulea, 1939; Thirumalachar, 1941; Kirulis, 1942; Rayss, 1943; Keener, 1954; and Barkai-Golan, 1959).

### THE PURPLE MOLD

Among the first fungi reported associated with white pine blister rust cankers was the purple mold, Tuberculina maxima. This Deuteromycete was described by Rostrup (1890). Tubeuf (1901) discussed the taxonomy and host range of the genus Tuberculina and reported T. maxima able to "destroy (control) blister rust of white pine". His observations reported in 1914 did not fulfill their expectations. Lechmere (1914) clarified the life history of T. maxima and considered it a typical example of double parasitism. He concluded that only the pycnia and aecia could be attacked; that the parasite cannot invade the pine tissue itself and destroy the rust mycelium and therefore could not be employed as a biological control. These conclusions were based on only a few observations; at least, no experimental data was given. Tubeuf (1930) and Rohmeder (1931) concluded that one does good by using T. maxima to combat the blister rust disease.

The purple mold was first reported in the United States by Weir and Hubert (1917) on native pine rusts in Montana. It was first found on blister rust in North America on western white pine at Daisy Lake on the coast of British Columbia in 1926 (Mielke, 1933). Until 1932 it had been found on this host only along the coast of British Columbia where it also occurred on native Cronartium comptoniae on lodgepole pine. In 1932 it was found on white pine blister rust for the first time in the United States at Newman Lake, near Spokane, Washington, (Mielke, 1933; Hubert, 1935a), where the blister rust was first found on pine in the Inland Empire in 1928 (the rust became established here and at other locations in the Inland Empire in 1923). In 1932 the purple mold was

found also in interior British Columbia on western white pine rust cankers (Mielke, 1933).

Although early observations on the purple mold's ability to control the rust on western white pine were not encouraging (Goodding, 1932; Hubert, 1932; Mielke, 1933), each of these early workers expressed the possibility that the mold could develop a strain that would be more virulent on the rust. In 1931, Hubert obtained a strain of T. maxima from Germany that he hoped would control the rust here as it had in some parts of Germany. He inoculated rust cankers at two localities in Idaho, but by 1934 this mold strain from Germany, as well as material obtained in British Columbia in 1931 and 1932, had given unsatisfactory control results (Hubert, 1935a).

The early work with T. maxima on the rust of western white pine, as well as the European work on other pines, demonstrated the ability of the purple mold fungus to parasitize rust cankers. In the Pacific Northwest, Goodding (1932) reported he found the purple mold "often completely destroying aecial production." Hubert (1932) stated, "Every canker showing purple mold gave evidence that aecial sporulation had been arrested." His concern was not with the effect of the parasite on the rust cankers, but with its apparent inability to spread to enough cankers to appreciably reduce rust damage. Mielke (1933) stated, "The parasite attacks both pycnia and aecia of the white pine blister rust. In British Columbia it has been observed that production of the aeciospores is greatly reduced when the aecia are attacked and that death of the bark over the cankered area often occurs in a few years." In Germany, Tubeuf (1930) found by inoculation that T. maxima suppressed formation of aecia and repeatedly led to the healing of the aecial stroma in pine.

In British Columbia, Mielke (1933) observed that pycnia may be attacked throughout the entire seasonal period of their development, production is reduced, and a pycnial zone attacked one season usually fails to produce aecia the following year. Spaulding (1929) observed in Europe, where T. maxima occurs, that pycniospores are not produced in great abundance. A study made at Oregon State University showed that an aqueous solution of the pycnial fluid is an excellent medium for the germination of T. maxima spores (Mielke, 1933). Because of the preference of purple mold on pycnia, he suspected that its effect on aecial production was indirect. He substantiated this by shaving off the outer bark of the parasitized portion of a stem canker and one month later observed T. maxima sporulating profusely on the cut surface. He interpreted this to demonstrate that the purple mold's mycelium invades the bark well beneath the pycnia and aecia on the surface. He believed that this may explain why this fungus appears to hasten the death of infected bark.

In a study of the fungi associated with blister rust cankers of western white pine, made in 1941 in the Inland Empire, Bingham and Ehrlich (1934) reported 24 different perfect and imperfect stages of fungi. Only one of

these, T. maxima, was considered to be parasitic on the rust fungus itself. The others were considered weak bark parasites or saprophytes which subsist on the weakened or dead bark of the blister rust cankers. These bark parasites starve out the rust by killing the cankered bark, upon which the rust fungus is dependent for its sustenance, while T. maxima parasitizes the pycnia and aecia of the rust fungus itself (Bingham and Ehrlich, 1943).

The published literature, unpublished observations by professional personnel, and our field and laboratory data from work initiated since 1965, indicate that the lilac fungus, T. maxima, is the most active biological agent having a definite and demonstrable pathologic effect on blister rust cankers. This imperfect fungus reduces the inoculum potential of C. ribicola by suppression of both pycnia and aecia production and inhibits growth and development of the cankers, which results in partial or, more rarely, complete inactivation of such cankers.

Results from a survey in 1965 (Kimmey, 1969) showed that T. maxima was distributed through the western white pine type in the Clearwater, Coeur d'Alene, Colville, Kanisksu, and St. Joe National Forests. The fungus was actively fruiting on 24.3 percent of the active lethal-type cankers. T. maxima fruits only on active cankers; it is not possible at present to ascertain accurately its past occurrence on cankers. Aecial production was reduced to 14.2 percent for the active lethal-type cankers and to 5.4 percent for the entire lethal-type canker population. This was not entirely the result of action of T. maxima, but a large percentage of it was undoubtedly associated with T. maxima infection, because the fungus was fruiting on 1.4 percent of the cankers producing normal aecia, 39.7 percent of the cankers producing partial aecia and 25.2 percent of active cankers producing no aecia. Our inability to recognize the presence of T. maxima in the absence of fruiting certainly masked the relation between this fungus and aecial production.

T. maxima has not been reported in association with uredial or telial sori of C. ribicola on Ribes spp. Tubeuf (1901) reported collections by several European workers (Gobi, Frank, Magnus, Schroter, and Rostrup) of Tuberculina spp. occurring on uredia and telia as well as spermogonia and aecia of several rusts. However, in all cases where enough information is provided to permit a check on the nature of the life cycle of the rusts, the collections of Tuberculina spp. reported in association with uredia and telia were on autoecious rusts, regardless of whether they were macrocyclic, demicyclic, or microcyclic. In these cases, infection by Tuberculina spp. may have occurred during pycnia and/or aecia production and remained throughout uredia and telia production. One collection listed by Tubeuf (1901) reports a species of Tuberculina on Darlucalium filum (Biv.) Cast., another imperfect fungus associated exclusively with the uredinales. Gobi (1885) reported Tuberculina spp. on Paris quadrifolia without finding a uredinales on the host plant. Undoubtedly a rust was present, Gobi just failed to detect it.

I have successfully isolated T. maxima from the discolored margin of "dormant" cankers collected from the field during the winter months (December-March). I know that T. maxima can overwinter on western white pine blister rust cankers as spores, sporodochia, and as mycelium within the pine cortex (Wicker and Wells, 1968). Continuous freezing for periods up to 19 months does not completely destroy spore viability. Thus, T. maxima is well adapted to overwintering in the imperfect state and viable inoculum is available, under natural conditions, for invasion of blister rust cankers any time the cankers are susceptible and the environment is favorable. Also, reinfection is not necessary on an annual basis. These data and our many field observations indicate that the mycelium of T. maxima does ramify throughout the cankered pine tissues and is not restricted to the aecia and pycnia as Tubeuf (1914a, 1914b) and Lechmere (1914) reported. T. maxima does not go beyond the rust invaded pine tissue and, thus, does not constitute a canker and subsequent threat to the tree itself.

T. maxima can only infect western white pine blister rust cankers during pycnia and aecia production. Non-fruiting cankers (juvenile or fruiting retarded) could not be infected even following scarification of the discolored canker tissue with carborundum (Wicker and Kimmey, 1967).

Data from artificial inoculations of blister rust cankers with spore suspensions of T. maxima both in the field and under controlled conditions in growth chambers (Wicker and Wells, 1970) show the incubation period to be two-seven weeks for both aecia and pycnia inoculations. The incubation period is defined as the elapse time between inoculation and subsequent spore production. Thus, the incubation period encompasses both the penetration and infection periods. It is quite evident that T. maxima is capable of completing its life cycle in a very short period.

T. maxima can be grown on a variety of artificial media. Investigations of the nutritional requirement (unpublished) show that the fungus can utilize numerous carbon and nitrogen sources. There are some it cannot use and still others, such as nitrite compounds, that may even be toxic. The fungus grows exceedingly well in pycnia fluid. This fluid is known to contain ammonia, 21 amino acids, inorganic phosphate, fructose, three acyclic sugar alcohols and inositol (Wicker et al., 1976). Growth of the fungus is favored by a high carbon content; C/N ratios of 20:1 and 25:1 (unpublished).

My investigations of the ecology of T. maxima have defined the temperature range. Spores germinate at 5°C with little or no additional growth. The thermal death point is between 25-30°C as no growth occurs at 30°C. The optimum temperature appears to be between 20-25°C and varies slightly according to the medium used. Our studies of pH are in process and so far we have grown the fungus on media from pH 3.0-8.0. The optimum is around pH 5.0.

During our field evaluations of cycloheximide and phytoactin as chemical controls for the blister rust disease (1965-1967), initial observations indicated that these two antibiotics were suppressing T. maxima at the concentrations used when applied directly to the canker surfaces. Our data from both field and laboratory studies confirm both antibiotics to be phytotoxic to T. maxima. When tested in vitro, cycloheximide completely inhibited T. maxima at 1-1000 ppm. Complete inhibition occurred with phytoactin at concentrations 70 ppm (Wicker, 1968). These data suggest that direct canker treatment using these two antibiotics may have been detrimental to blister rust control rather than just ineffective (Leaphart and Wicker, 1968; Wicker, 1968).

Results from histological study of the pine-rust-T. maxima association (Wicker and Woo, 1969; 1973) show that the effects of T. maxima on the rust infected tissues of pine seedlings and tissue cultures are rapid, lethal, and result in structural degradation. Walls, cytoplasm, and nuclei of rust-infected pine cells are hydrolyzed as the mycelium of T. maxima invades them. We observed haustoria, hyphae, and sporogenous cells of the rust to lyse as the pine cells were destroyed. T. maxima does not attack the rust-free pine tissues in vivo. However, it can establish parasitic and pathogenic relationships with rust-free pine bark tissues growing in vitro. We conclude from these studies that destruction of rust parasitized pine cells by T. maxima results from the controlled action of several enzymes (Wood, 1960) secreted by the lilac fungus. This enzymatic action is directed at the rust parasitized pine cells and not at the rust organism per se. Thus, the mode of action of T. maxima is the enzymatic destruction of the food source vital to the survival of the obligate rust organism.

Wicker and Shaw (1968) listed six attributes essential to the success of a biological control agent: a) distribution which coincides with that of the target pathogen, b) ecologic amplitude sufficient to ensure persistence within its susceptible range, c) production of abundant inoculum for establishment of epiphytotics, d) high infectivity, e) high virulence, and f) an efficient mode of action for curtailing development of target disease. I would rate T. maxima positive for the first three attributes and negative for the later three. The purple mold has delayed damage by blister rust and prolonged the life of afflicted white pines but it has not controlled the disease.

Recent surveys (Powell, 1971a; 1971c), mycological collections (Powell, 1971b; 1972), and personal observations indicate that T. maxima is well established in western North America on such rusts as Cronartium ribicola, C. comandrae, C. comptoniae, Peridermium harknessii, and P. stalactiforme. There are undoubtedly other uredinaceous hosts for this fungus in North America.

Tuberculina maxima has been observed infrequently in eastern North America (Hubert, 1935a; Hedgecock, 1935). In 1966, I provided

Dr. W. R. Phelps, Rhinelander, Wisconsin, cultures of an isolate of the purple mold from the western United States. This isolate was used to successfully inoculate C. ribicola cankers on Pinus strobus growing in the vicinity of Rhinelander. I personally observed these inoculations in August, 1967 and confirmed that T. maxima was established within the cankers. Since that time, the frequency of the purple mold is reported to be increasing in Wisconsin and Minnesota on native conifer rusts. (Bergdahl, personal communications).

Likewise, in 1967, I provided Dr. J. W. Koenigs, Research Triangle Park, N.C. with cultures of a T. maxima isolate from Idaho to be used for inoculating C. fusiforme galls on southern pines. I have no information to confirm that the inoculations were ever made and, if they were, whether they were successful. However, I am not surprised that the purple mold was recently reported infecting fusiform rust galls on southern pines in North and South Carolina (Kuhlman and Miller, 1976).

Tuberculina maxima is known to infect C. ribicola cankers on white pines in Japan (Yokota, personal communications). However, I did not observe the fungus to occur on the island of Hoppaido during my excursions there in 1974.

#### OTHER FUNGI AND BACTERIA

The possibility that biological components of the microflora of blister rust cankers, other than T. maxima, may have a pathological effect on the canker has not overlooked. However, investigation of such has attracted even less attention than has T. maxima. While several fungi are reported to inhabit blister rust cankers (Tubouff, 1910; Posey and Gravatt, 1917; Colley, 1918; Rhoads, 1920; Snell, 1929a, 1929b; Goodding, 1932; Wollenweber, 1934; Hubert, 1935a; Bingham and Ehrlich, 1943) information on the nature of their association with such cankers is essentially nil. There is a very notable void of reports of fungal inhabitants of blister rust cankers, other than T. maxima, from Europe and Asia.

On each of 13 sample strips examined during their study, Bingham and Ehrlich (1943) estimated 0-14 percent of the rust-threatened trees had been saved through intercession of the canker fungi. They believed the smallness of these percentages was due, in part, to the intense pine infection and the small size of the reproduction of the selected strips, where the proportion of stem cankers was particularly high. Each such stem-cankered tree constituted a threatened tree, the deliverance of which could not be effected. This fact was well demonstrated in two strips where more than 70 percent of the potentially damaging branch cankers were parasitized and judged dying but where only 0 or 2 percent of the threatened trees could be considered saved by the parasitic canker fungi. Where trees are larger or the rust infection not so intense, they

concluded the less numerous direct stem infections should increase the effectiveness of the canker fungi in saving rust-threatened trees. Their data showed that the percentage of canker parasitism is dependent more on age and stage of cankers than on intensity of pine infection.

On their 13 sample strips, Bingham and Ehrlich (1943) found aecial sporulation reduced 5 to 60 percent by canker parasitism. They found a high and significant degree of direct correlation of aecia reduction with the percentage of cankers parasitized. Volume of aeciospores is reduced more by parasitism than is percentage of aecia; as 1-year sporulating cankers, which produce relatively small volumes of spores, were parasitized 32.5 percent while 3-year sporulating cankers, which produce relatively large volumes of spores, were parasitized 57 percent.

In recent years, field observations and data reported by several workers indicate an increasing degree of natural inactivation of blister rust cankers on western white pine growing in the Inland Empire. The accumulated evidence that the microflora (primarily fungi) which inhabit blister rust cankers may have an adverse effect upon growth and development of such cankers, either directly or indirectly, supports this conclusion. Williams (1972) lists 49 different fungi inhabiting western white pine blister rust cankers in the Inland Empire with both perfect and imperfect stages of some being commonly encountered. Some of these are mere epiphytes, some are saprophytes, some are parasites, and a few are pathogens.

I have collected and isolated in pure culture ca. 120 fungi and a dozen or so bacteria from rust cankers in Europe and some 50 fungi and another dozen bacteria from rust cankers in Japan. However, to date, these organisms have not been tested on blister rust cankers on western white pine.

#### AGENTS OTHER THAN FUNGI AND BACTERIA

Organisms other than fungi and bacteria are known to inhabit and/or feed upon white pine blister rust cankers (Hubert 1935b; Furniss et al 1972). Numerous arthropods feed upon the rust structures or the rust-infected pine tissues. Rodents, such as squirrels and mice, feed upon infected pine tissues. All these agents effect some degree of biological control of the rust and it is important that their ecological roles be determined and documented.

#### OTHER RUSTS

Biological agents associated with native conifer rusts have received even less attention than those of white pine blister rust. Within the last decade, several excellent papers have reported on biological agents

associated with rust cankers on hard pines in western Canada (Powell 1971a, 1971b, 1971c, 1971d, 1971e, 1972, 1974; Powell, et al, 1972). Byler, et al (1972a, 1972b) reported on the occurrence and effects of fungi on western gall rust in California.

#### CONCLUSION

Within the past decade, America has experienced an uprising of public interest and concern relative to the utilization of renewable natural resources, particularly, her forests. Consequently, social, economic, and political demands for maximum and efficient use of forest goods and services to ensure the greatest public benefits have increased proportionately. Reduction of losses due to destructive agents, such as diseases, is of major significance in meeting these demands.

It should be obvious by now - if, indeed, we learn from past mistakes - that the application of single, specific measures to reduce losses from disease is seldom effective. What is needed for the future, in my opinion, are numerous and varied protective and preventative measures that are integrated into forest management systems based on the ecology of the forest area being managed.

I believe it is the responsibility of research forest pathologists to develop these protective and preventative measures and to work, hand-in-hand, with disease control specialist and forest land managers to integrate such measures with management systems.

If the above philosophy is rational and has merit, then why do we continue to evaluate biological control measures with the premise that they, alone, must effect the desired level of control before they are acceptable?

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TREE GROWTH SUPPRESSION IN RESPONSE TO  
LONG-TERM LOW LEVELS OF O<sub>3</sub>, NO<sub>2</sub>, AND/OR SO<sub>2</sub>.

Lance W. Kress

Air pollutants usually occur in combination with one another and at rather low levels in the ambient atmosphere. The National Ambient Air Quality Standards (NAAQS) have been established based primarily on reports of single pollutant effects (only California standards have considered O<sub>3</sub> + SO<sub>2</sub> interactions). When setting the NAAQS little attention has been paid to the potential interactions of pollutants primarily because little information regarding the effects of interactions has been available. The lack of adequate technology for handling and working with certain pollutants in combination has been a major stumbling block, but recently instrumentation has become available that could be used in support of research regarding the effects of several pollutant interactions to vegetation.

Another major void in the knowledge of pollutant effects to plants has been the failure to deal with the effects of long-term low-level exposures. It is important to consider that the exposures must occur daily without weekend breaks. Under ambient conditions, plants are usually exposed seven days per week, and sometimes pollutant levels are even higher on weekends.

When this investigation was undertaken, the author was aware of only one researcher who had exposed plants to controlled pollutant levels for long-term (seven days per week) low-level fumigation. Barnes (1, 2) exposed several pine species to low levels of O<sub>3</sub> continuously for up to 22 weeks and measured photosynthesis, respiration, and some biochemical compounds. There were no reports of long-term low-level effects to vegetation of mixtures of three pollutants.

Loblolly pine (*Pinus taeda* L.) and American sycamore (*Platanus occidentalis* L.) were selected as the plant species for this study. Full-sib families of loblolly pine and half-sib families of sycamore were available.

The objectives of the study were: to determine if relatively ambient levels of O<sub>3</sub>, NO<sub>2</sub>, and SO<sub>2</sub> could cause significant growth suppressions and foliar symptoms on sensitive and tolerant families of loblolly pine and American sycamore; and to evaluate the effects of three pollutants in combination in long-term exposures --- a basic study that had never been accomplished for any crop.

## MATERIALS AND METHODS

Sensitive and tolerant families of loblolly pine and sycamore were chosen based on earlier  $O_3$  screening studies. Seedlings were started from seed on a staggered planting schedule so as to have uniform aged trees for each of three exposure groups. The seedlings were planted four per pot in four inch pots containing a 2:2:1 weblite:vermiculite:peat moss planting medium. When the pines were approximately 2 weeks old and the sycamore approximately 1 week old, they were exposed to the pollutants in one of three exposure series:

- exposure number 1 - Control,  $O_3$ ,  $SO_2$ , or  $O_3 + SO_2$ ;
- exposure number 2 - Control,  $O_3$ ,  $NO_2$ , or  $O_3 + NO_2$ ; or
- exposure number 3 - Control,  $O_3$ ,  $O_3 + SO_2$ , or  $O_3 + SO_2 + NO_2$ .

Each exposure series ran for 28 consecutive days:

- exposure number 1 - Jan. 26 to Feb 21;
- exposure number 2 - Feb. 25 to Mar. 23; and
- exposure number 3 - Mar. 30 to April 26, 1976.

The exposure series were repeated in 1977 on new seedlings. The plants were exposed for 6 hours per day. The pollutant levels used singly and in combination were 5 pphm  $O_3$ , 14 pphm  $SO_2$ , and 10 pphm  $NO_2$ .

Prior to exposure, during the non-exposure portions of the day, and after exposure, the plants were maintained in a charcoal-filtered greenhouse, under a 14 hr photoperiod. Lighting of 21,000 lux were provided by high pressure sodium lamps. Each pot received 1 gm of osmocote fertilizer and was watered routinely.

The monitors used were: Bendix chemiluminescent for  $O_3$ , calibrated by the 1% neutral buffered KI method; Bendix chemiluminescent  $NO_2$ , calibrated by a known level of  $NO$ ; and Bendix flame photometric total sulfur analyzer for  $SO_2$  that was calibrated with an  $SO_2$  permeation tube.

The exposure chambers employed a single-pass air system, drawing in charcoal-filtered air having a chamber residence time of two minutes. The pollutants were injected into the intake lines just prior to their entry into each chamber. Pollutant concentrations were maintained at the desired levels by monitoring at plant height and adjusting the flow of pollutant into the chamber. Lighting for the chambers was 30,000 lux provided by high pressure sodium lamps. Environmental conditions were 25-30°C and 70-85% RH.

The seedlings were evaluated for foliar symptoms prior to the exposure, at the termination of the exposure, and at two weeks later. Height growth of the seedlings was measured at weekly intervals.

## RESULTS - LOBLOLLY PINE

Significant differences in height growth were noted between some treatments, and between the two families within some treatments. Foliar damage was light, but significant differences in foliar symptom response between treatments and between families within treatments were also noted. While the sensitive family exhibited greater foliar damage and greater growth suppression than the tolerant family, the actual amount of foliar damage was not necessarily correlated with growth suppression when comparing any two treatments, especially within a family.

The predominant symptoms noted were mottle and chlorotic spot, but the amounts of each symptom varied with treatment and family. The sensitive family (4-5x523) exhibited the mottling symptom for all pollution treatments. In 1976, the chlorotic spot symptoms were common (but still secondary to the mottling symptom) mostly on the trees exposed to the single pollutant treatments. In 1977, the chlorotic spot symptoms were predominant on the single pollutant exposures, while the mottling symptoms were predominant only on the combined pollutant exposures.

Overall, the average foliar damage (SSI) for a family never exceeded 1% of the potential maximum (approximately 40,000), although individual trees had up to 5% damage.

Comparison of the tree height growth in similar treatments across exposure groups demonstrated a tendency for height growth to increase from exposure groups 1 to 3, but the differences were not significant at exposure termination for either family. Therefore, Table 1 was derived by averaging all the similar treatments and arriving at average growth suppressions for particular treatments versus the control treatment or the  $O_3$  alone treatment. The  $O_3 + SO_2$  and  $O_3 + SO_2 + NO_2$  treatments consistently resulted in significantly less growth than either the  $O_3$  alone or the control treatments. The sensitive family always exhibited greater growth suppression than the tolerant family in the  $O_3 + SO_2 + NO_2$  treatments.

## RESULTS - AMERICAN SYCAMORE

Significant growth suppressions resulted from some treatments in the almost complete absence of pollution induced foliar symptoms. The effects were most pronounced in the pollutant combination treatments, but even  $O_3$  alone caused significant growth suppression in some cases. At the level used (10 ppm),  $NO_2$  consistently had a stimulatory effect on sycamore height growth. American sycamore also demonstrated a capacity for recovery of the pollution induced height growth suppression after removal of the pollution stress.

### Foliar Symptoms

A few foliar symptoms were noted in 1976. Leaf chlorosis and distortion was common on all trees including controls. It was probable

Table 1. Height growth of loblolly pine seedlings (exposed to 5 ppm O<sub>3</sub>, 14 ppm SO<sub>2</sub>, and 10 ppm NO<sub>2</sub> singly and in combination for 6 hours per day, 28 consecutive days) expressed as a percent of either the control trees or the O<sub>3</sub> alone exposed trees.

1976 Loblolly Pine Seedling Growth as Percent of Control During Four Weeks Exposure to:						
Family	O <sub>3</sub> <sup>1</sup>	SO <sub>2</sub>	NO <sub>2</sub>	O <sub>3</sub> + NO <sub>2</sub>	O <sub>3</sub> + SO <sub>2</sub> <sup>2</sup>	O <sub>3</sub> + SO <sub>2</sub> + NO <sub>2</sub>
4-5 x 523	95	84*	105	90	78*	70*
14-5 x 517	98	113	94	90	86*	88*

<sup>1</sup> Average of 3 O<sub>3</sub> treatments.

<sup>2</sup> Average of 2 O<sub>3</sub> + SO<sub>2</sub> treatments.

\* Significantly different from the control at the .05 level.

Loblolly Pine Seedling Growth as Percent of O <sub>3</sub> Exposed Plants During Four Weeks Exposure to (Average of 1976 and 1977 Data):					
Family	SO <sub>2</sub>	NO <sub>2</sub>	O <sub>3</sub> + NO <sub>2</sub>	O <sub>3</sub> + SO <sub>2</sub>	O <sub>3</sub> + SO <sub>2</sub> + NO <sub>2</sub>
4-5 x 523	83	105	100	78*	74*
14-5 x 517	100	111	99	84*	85*

\* Significantly different from the O<sub>3</sub> treatment at the .05 level.

that the symptoms noted were not actually pollution induced. Typical oxidant stipple or typical SO<sub>2</sub>-induced interveinal necrosis was never noted. No foliar symptoms were noted for the 1977 exposures.

The sycamore height growth response to various treatments is shown in Figure 1. In 1976 the O<sub>3</sub> treated trees grew significantly less than the controls and as another pollutant was added there was a further significant reduction in height growth. The 1977 data match 1976 except for the controls. In 1976 all treatments were in identical chambers (square), but in 1977 the controls were in a different chamber type (cylindrical) than the 1976 pollution treatments. When all treatments were in the cylindrical CSTR chamber, the O<sub>3</sub> exposed plants grew less than the controls in 1977 also (Figure 2).

## DISCUSSION

The data indicated that long-term low-levels of O<sub>3</sub>, NO<sub>2</sub>, and SO<sub>2</sub> in combination were capable of causing significant growth suppressions in loblolly pine with minimal foliar injury, and significant growth suppressions in American sycamore in the absence of foliar symptoms. The pollutant levels used were below the National Ambient Air Quality Standards for each pollutant. The study also provided positive indications that specific families of loblolly pine were sufficiently stable to serve as bioindicators of ambient pollution levels.

The control treatments (in relation to the pollutant treatments) exhibited significant variation from 1976 to 1977. The controls for 1976 were in a square exposure chamber immediately following a pollution treatment, but for 1977, they were in a cylindrical chamber at the same time that the pollutant treated trees were in the square chambers. Figure 1 demonstrated the difference very well. The control trees were the largest of the treatments in 1976, but they were smaller than both the O<sub>3</sub> alone and O<sub>3</sub> + SO<sub>2</sub> treatments in 1977. The same phenomenon was noted with the pine seedlings. The data in Figure 2 demonstrated that when all the treatments occurred in the cylindrical chambers, the control plants were taller than the O<sub>3</sub> alone plants. Therefore, the growth differences between the control treatments from year to year seem to be related to the difference between the square and round chambers. The cylindrical chambers were generally up to 5C cooler and 5% RH higher than the square chambers, but the major difference was probably in air exchange. The total flow rate was set to be the same in both sets of chambers, but since the cylindrical chambers were about twice as large as the square chambers, the residence time for air in the cylindrical chambers was about twice as long. Whether or not that was the reason for the difference is unknown. It is possible that CO<sub>2</sub> levels were depleted in the slower moving air (by the plants) and thus became a limiting factor for photosynthesis.

Previous screening studies had demonstrated that the pines were much more sensitive to O<sub>3</sub> in terms of foliar symptom response in early July than at other times of the year, given the same aged tissue (5). In this

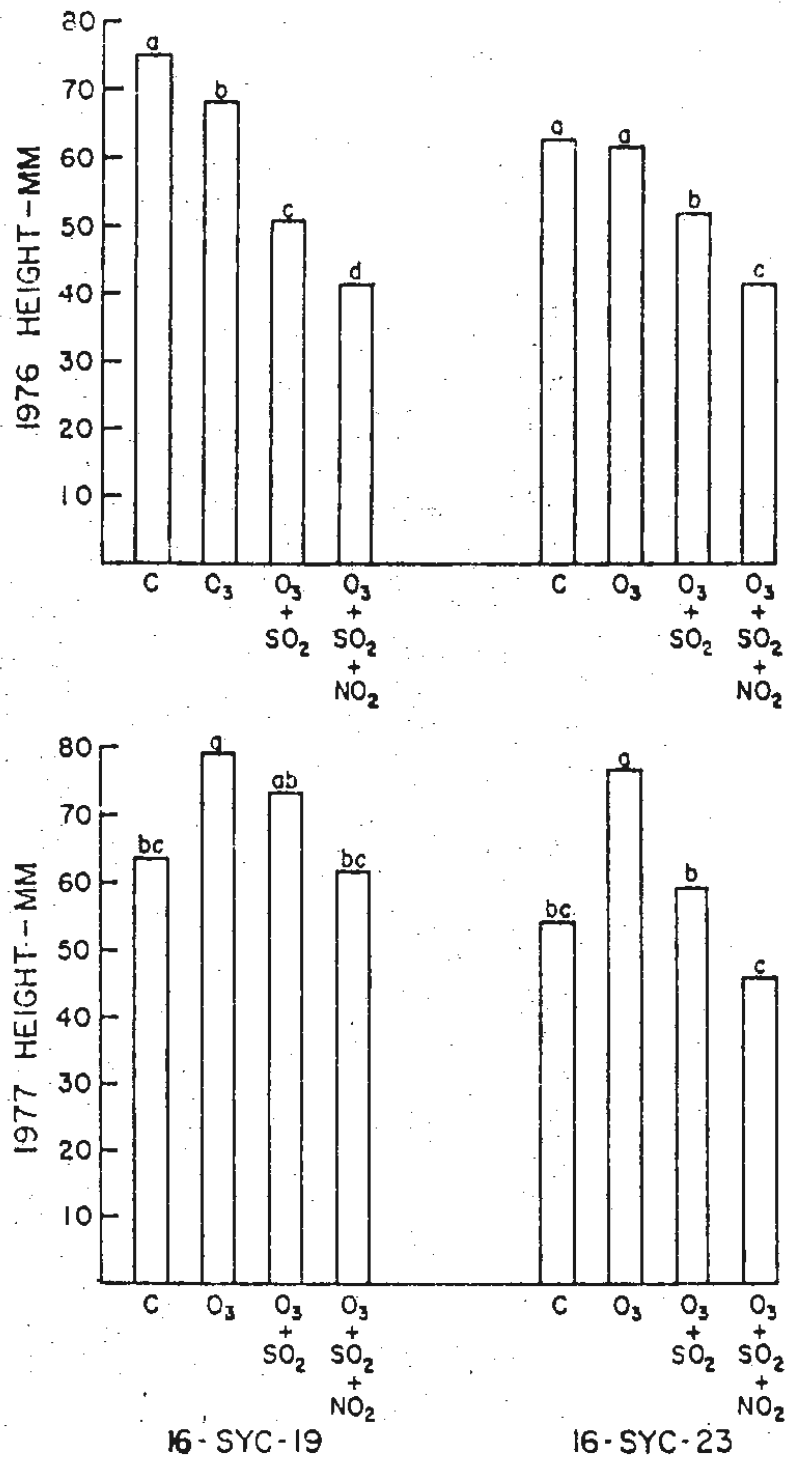


Figure 1. Total height of both families (sensitive - 16-Syc-19 and tolerant - 16-Syc-23) of sycamore exposed to various treatments at the end of the 4 week exposure period. The comparable 1976 data are shown above the 1977 data. All pollution treatments were in square chambers. The 1976 controls were in a square chamber 6 hours following the pollution treatments, and the 1977 controls were in a cylindrical chamber at the same time as the pollution treatments.

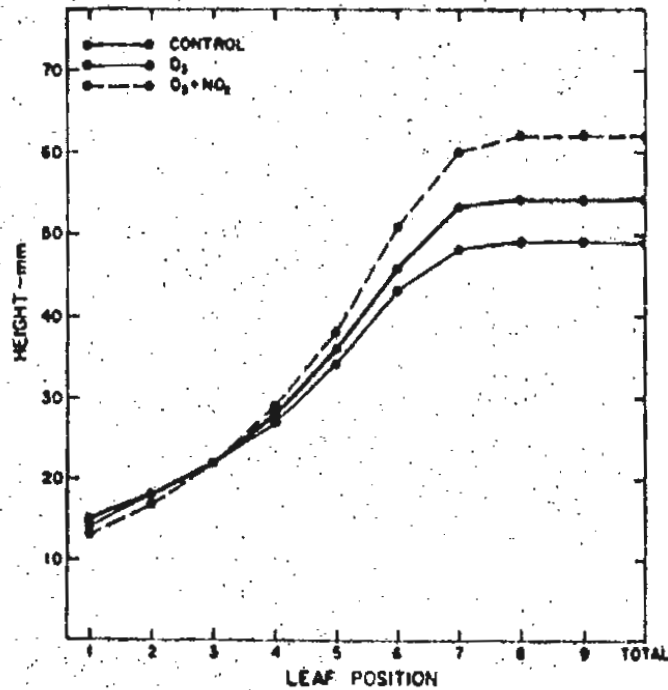
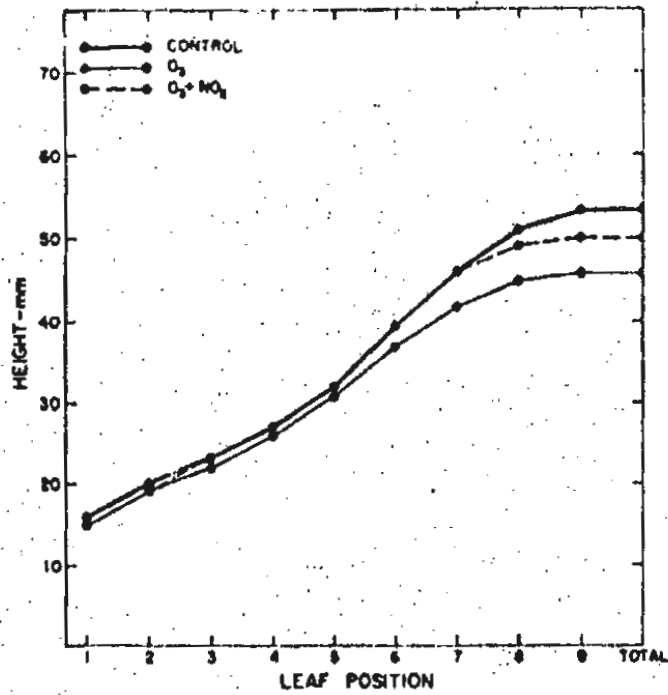


Figure 2. Height to first 9 leaves and total height of a sensitive (16-Syc-19 - top) and a tolerant (16-Syc-23 - bottom) sycamore family exposed to various treatments of 5 ppm O<sub>3</sub>, 14 ppm SO<sub>2</sub>, and 10 ppm NO<sub>2</sub> after 28 consecutive days of exposure 6 hr/day in 1977. All treatments were in cylindrical (CSTR) chambers.

study, the most significant differences occurred in exposures that occurred in April and May versus February and March. Based on these results more significant growth reductions and greater foliar damage could have been expected had the exposures occurred in June and July.

Significant growth suppressions (up to 45%) for sycamore and 30% for loblolly pine were noted in this study, even though the pollutant levels used were below the NAAQS for each pollutant. The results of studies like this one must be considered when the NAAQS are re-evaluated. Pollutants naturally occur in combination and the potential effects of these combinations are probably more damaging than anyone ever realized. A 45% loss for any endeavor (even if it has an exceptional profit margin) would hardly be acceptable. The profit margin for most forest industries is rather low, and possibly air pollution growth impact has been a major reason.

The results of this study provide considerable evidence in favor of the theory of hidden damage. Previous work has demonstrated hidden damage: Treshow et al. (7), Barnes (1,2), Tingey et al. (6), Keller (4), and Houston and Dochinger (3). However, none of the previous reports have examined growth under such closely controlled exposure conditions and reported such significant growth suppressions in the total absence of foliar symptoms. Hidden damage has been demonstrated in a convincing manner by these results. Hidden damage should be considered as fact rather than theory.

It would be extremely difficult but interesting to carry these results forward to determining the actual growth loss of forest trees that must be occurring due to ambient levels of air pollution. As mentioned previously, the entire forest tree improvement program in the South is based upon realizing a 5% increase in yield; this study has demonstrated that up to a 30% reduction in pine growth and a 45% reduction in sycamore growth could be occurring due to the interaction of three major air pollutants.

This study is the first to involve three major pollutants in long-term (7 days per week) low-level exposures, and to examine the growth impact of such exposures. These results must be considered in the establishment of the NAAQS. It is unrealistic to base the standards on single pollutant effects when plants and people are constantly exposed to several pollutants at the same time. These results must also be considered when evaluating the economic effects of air pollution. Growth impact due to invisible damage has the potential to be devastating. Trees appear to be less sensitive than crop plants to most air pollutants in terms of visible foliar damage. However, due to their perennial nature, they may be much more sensitive to the here-to-fore unappreciated invisible damage.

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## HERITABILITY OF OXIDANT SENSITIVITY IN LOBLOLLY PINE

Lance W. Kress

In studies of environmental effects (such as air pollution stress) on plants, minimization of plant to plant genetic variation must be considered. This is especially important in studies where a relatively small number of plants are to be examined. Substantial plant to plant variation within treatments results in a high coefficient of variation and can effectively mask any differences between treatments. Two ways of minimizing genetic variation are cloning (by grafting or rooting cuttings) and by controlled pollinations to generate full-sib families.

The minimization of genetic variation is of paramount importance when attempting to develop a bioindicator system for detection and quantification of ambient levels of air pollution. A bioindicator system as suggested by Berry (2) would supplement and in part replace mechanical monitoring instruments, and biologically demonstrate the effectiveness of pollution abatement programs. However, the symptom responses must be under adequate genetic control to allow good repeatability of the responses.

The primary objective of this study was to screen several full-sib families of loblolly pine for sensitivity to  $O_3$  or  $NO_2$  in order to identify a sensitive and a tolerant family for use in subsequent field studies utilizing open-top chambers at the Radford Army Ammunition Plant (9). A second objective was to attempt to determine whether or not tolerance or sensitivity of loblolly pine to air pollution was a heritable characteristic.

### MATERIALS AND METHODS

Seeds of 18 full-sib families of Pinus taeda were obtained from Dr. Bruce Zobel of the North Carolina State University - Industry Cooperative Tree Improvement Program. The seeds were sown in a 2:2:1 by volume weblite® (an expanded shale product of the Webster Brick Company, Roanoke, Virginia):vermiculite:sphagnum peat moss potting mix. As the seeds germinated, the seedlings were transplanted four/pot into .45 liter plastic pots with the same potting mix. The trees were maintained in a greenhouse supplied with charcoal-filtered air. Supplemental lighting was provided on a 14 hr photoperiod with high pressure sodium lamps which produced 21,000 lux (330 microeinsteins  $m^{-2} sec^{-1}$ ). Throughout the study, the trees were watered daily to saturation. The seedlings were fertilized with a single application of one gram of osmocote spread uniformly over the potting medium surface in each pot 2 to 3 days following transplanting.

During the summer of 1975, and the summer and fall of 1976, four trees of each family were exposed to varying amounts of  $O_3$  or  $NO_2$ . There was a single set of control plants for each of the two pollution treatments occurring on a single day. To determine if secondary needle response to  $O_3$  was relatively similar (among families) to the response of primary needles on the same tree the previous year, some trees were exposed in 1975 and again in 1976.

Indoor exposure chambers were constructed as per the specifications of Heck et al. (6). The chambers were 72 cm x 72 cm x 75 cm high, drew charcoal-filtered air, and employed a single pass air system having a chamber residence time of 1.7 minutes. The pollutants were injected into the intake air stream prior to its entry into the chamber. Pollutant concentration varied less than one part per hundred million (pphm) (at 25 pphm) at plant height in the chamber. Ozone was generated by passing oxygen around an ultraviolet lamp in a water jacketed 3" x 9" glass cylinder. The ozone enriched air was carried to the three fumigation chambers via teflon tubing and rotometers controlled the flow. The air inside each chamber was sampled continuously through a tube placed at plant height. Ozone was monitored in 1975 with a Mast Oxidant meter (Mast Development Co., Davenport, IA, 52803) calibrated by the 1% neutral buffered potassium iodide (NBKI) technique (14). In 1976,  $O_3$  was monitored by a Mast Oxidant meter and a Bendix model 8002 chemiluminescent ozone analyzer (Bendix Process Instruments Division, Lewisburg, W. Va., 24970), and again calibrated by the NBKI technique. Nitrogen dioxide was obtained as bottled gas diluted in nitrogen (1.09%  $NO_2$ ), and delivered to each chamber via teflon tubing and controlled with a rotometer. The  $NO_2$  concentration was monitored with a Mast Oxidant meter calibrated by the NBKI technique (approximately 10% efficient for  $NO_2$ ). The pollutant concentrations in the chambers were maintained by monitoring at plant height and adjusting the flow rotometers. The  $O_3$  and  $NO_2$  levels were additionally spot checked occasionally during fumigation with the NBKI technique directly.

Lighting for the chambers was 28,000-30,000 lux (420-460 microeinsteins  $m^{-2} sec^{-1}$ ) at plant height provided by high pressure sodium lamps. Temperature during fumigations generally was 26-29C, but varied from 24-31C depending on season. Relative humidity was generally 70-80%, but varied from 50-90%. Temperature and RH were generally higher in the summer months.

The trees were exposed beginning at approximately 0800, and prior to and after fumigation they were maintained in the greenhouse. During all phases of the study, the plants within any treatment replication were treated as uniformly as possible. Placement on greenhouse benches and space assignment within the exposure chambers was completely random. Control plants were treated identical to the exposed plants except that no pollutants were injected into the chamber.

Foliar symptom evaluations were made prior to, immediately following, and 1, 7, 14, and 21 days following exposure. The control trees were examined first in each treatment, and symptoms (generally none) noted were considered to be not pollution related. When the same type symptom was noted on exposed trees it was also considered to be not pollution related, unless the intensity of the symptoms was drastically greater.

The foliar symptoms noted were assigned the following numerical value in an attempt to place an estimate of severity to the plant.

- 0 No symptom
- 1 Chlorotic spot
- 2 Mottle
- 3 Chlorotic band
- 4 Chlorotic tip
- 5 Necrotic spot
- 6 Necrotic band
- 7 Necrotic tip

The severity value was multiplied by the percent of the needle affected. This yielded a symptom severity index (SSI) for each needle. In cases where more than one symptom occurred on a needle, the one providing the highest SSI was recorded. Each primary needle was evaluated, and each fascicle of secondary needles was evaluated. The three needles within a fascicle were generally uniformly damaged, but in cases where they weren't, the highest SSI value was recorded for the entire fascicle. The SSI's were summed for all needles on a tree and divided by the total number of needles (symptomatic and asymptomatic) on the tree to yield an average tree SSI. The possible maximum average SSI was 700 and was dependent on needle damage severity and the percent of total needles damaged.

An analysis of variance table (ANOVA) to test for differences in mean SSI values between families in each treatment was generated using the Standard F-test as performed by the Statistical Analysis System (SAS) (1). The results of these ANOVA tests were then used for the Duncan's New Multiple Range Test to determine which families were significantly different in each treatment at the 0.05 level.

## RESULTS

The predominant symptom of  $O_3$  or  $NO_2$  exposure was a pigmented mottle that was usually tan in color, but occasionally it was reddish or purple. Mottling occurred both abaxially and adaxially, but was more intense on the adaxial surface. If the mottle was strictly chlorotic, it was classed as varying percentages of chlorotic spot.

Tip necrosis was common only in the 15 and 25 pphm treatments. Severely damaged trees had over half of the needles affected with some having half of the needles 100% necrotic.

The average tree SSI's for 18 families in response to 8 treatments in 1975 and 2 treatments in 1976 are listed in Table 1. Except for both replications of 10 pphm O<sub>3</sub> and 20 pphm O<sub>3</sub>, family 6-13x2-8 always exhibited the greatest amount of foliar symptoms. In all cases, family 6-13x2-8 sustained greater damage than families involving parent tree 504 (501x504, 529x504, and 540x504), and in all but the 10 and 20 pphm exposures, the difference was significant. In both replications of 15 pphm O<sub>3</sub> and the 9/3/76 25 pphm O<sub>3</sub> treatments, 6-13x2-8 was significantly more sensitive than the remaining 17 families. On 10/14/76 family 6-13x2-8 was significantly more sensitive than 16 of the 17 remaining families.

The objective of re-exposing trees in 1976 was not only to determine if family responses were consistent, but also to determine if individual tree responses within families were consistent. Table 2 presents the average tree SSI values for individual trees of 7 of the 18 families re-exposed to 25 pphm O<sub>3</sub> in 1976 compared to the 1975 responses to 20 pphm O<sub>3</sub>. The seven families were selected to illustrate that within some families the trees responded similarly relative to each other from year to year, while in some other families they did not.

The results of the NO<sub>2</sub> fumigations were inconclusive since few symptoms developed on any of the families. Within families 6-13x2-8 and 4-5x523 in the 7/10/75 replication of 50 pphm, one tree in each family accounted for over 95% of the average tree SSI. Two out of the four trees in each family had no symptoms at all. However, on the limited data available, it appeared as though 6-13x2-8 was relatively sensitive to NO<sub>2</sub> (as it had been to O<sub>3</sub>) and that 540x504 was relatively tolerant to NO<sub>2</sub> (as it had been to O<sub>3</sub>).

### Seasonal Variation

An appreciable variation in the response of the trees occurred with time of year. As indicated in Table 3, trees were most sensitive in early July. Damage in May and early June or late July was considerably less. The trees had been produced by sowing seed on a staggered schedule so that tree age over the different exposures was relatively uniform.

## DISCUSSION

The data indicate that it is feasible to use sensitive and tolerant families of loblolly pine as bioindicators of ambient air pollution. Based on foliar symptoms in response to O<sub>3</sub>, there is a good separation between the most sensitive and the most tolerant families when either primary or secondary needles are evaluated. While the NO<sub>2</sub> screening data are inconclusive, it does appear that some families sensitive or tolerant to O<sub>3</sub> are also sensitive or tolerant respectively to NO<sub>2</sub> and additional data indicate that the same is true for SO<sub>2</sub> (9). The finding that sensitivity and tolerance may be heritable should warrant further study into the feasibility of selection or breeding programs.

Table 1. Average tree symptom severity index (SSI) for 18 families of loblolly pine exposed to 15, 20, or 25 ppm O<sub>3</sub> for 8 h

Family	15 ppm		25 ppm		20 ppm		20 ppm		25 ppm		25 ppm		Average Tree SSI (max. 700)	
	7/2/75	7/2/75	7/8/75	7/8/75	7/14/75	7/22/75	7/14/75	7/22/75	9/3/76 <sup>1</sup>	10/14/76 <sup>1</sup>	9/3/76 <sup>1</sup>	10/14/76 <sup>1</sup>	9/3/76 <sup>1</sup>	10/14/76 <sup>1</sup>
2-40 x 2-8	14 b <sup>2</sup>	223ab	3 b	372a	16 b	2a	0a	0a	19 bc	5 c	57	5 c	57	
6-13 x 2-8	175a	245a	299a	376a	11 b	1a	0a	0a	46a	18a	103	18a	103	
503 x 4-18	0 b	94abc	1 b	23	1 b	0a	0a	0a	13 bc	1 c	12	1 c	12	
524 x 6-10	1 b	4 c	11 b	304ab	10 b	8a	0a	0a	7 bc	15ab	32	15ab	32	
501 x 504	0 b	5 c	13 b	1 e	5 b	0a	0a	0a	7 c	7 c	5*	7 c	5*	
529 x 504	1 b	15 c	6 b	7 e	4 b	0a	0a	0a	3 c	2 c	4*	3 c	4*	
540 x 504	1 b	64 c	0 b	32	0 b	0a	0a	0a	1 c	3 c	9*	1 c	9*	
6-7 x 508	2 b	9 c	2 b	27	0 b	0a	0a	0a	14 bc	3 c	6*	3 c	6*	
500 x 508	0 b	3 c	2 b	116	11 b	0a	0a	0a	22 b	13 b	17*	2 c	17*	
507 x 508	0 b	74 bc	5 b	135	3 b	1a	0a	0a	27 b	3 c	23*	2 c	23*	
525 x 511	0 b	117abc	20 b	244abc	1 b	0a	0a	0a	7 bc	2 c	33	7 c	33	
526 x 517	0 b	77 bc	2 b	70	3 b	0a	0a	0a	16 bc	7 c	17*	7 c	17*	
531 x 517	1 b	1 c	0 b	243abc	16 b	0a	0a	0a	10 bc	2 c	24	2 c	24	
14-5 x 517	0 b	13 c	32 b	72	0 b	0a	0a	0a	1 c	2 c	10*	1 c	10*	
4-18 x 523	1 b	107abc	2 b	29	59a	1a	0a	0a	1 c	6 c	18	6 c	18	
4-5 x 523	0 b	90 bc	20 b	57	4 b	0a	0a	0a	13 bc	4 c	17*	4 c	17*	
533 x 523	12 b	83 bc	8 b	155	21 b	4a	0a	0a	11 bc	1 c	26*	11 bc	26*	
546 x 523	0 b	112abc	2 b	177	1 b	0a	0a	0a	19 bc	4 c	28	19 bc	28	
Tree Age (wks)	3	3	3-4	3-4	3-4	5	3-4	5	8 <sup>5</sup>	12 <sup>5</sup>		8 <sup>5</sup>	12 <sup>5</sup>	
No symptoms	4 <sup>3</sup>	0	0	D	1	2	11	9	0	D		0	D	
Prob. > F	.0001 <sup>4</sup>	.0113	.0001	.0001	.0001	.1189	.5929	.6999	.0001	.0001		.0001	.0001	

\* Except for both reps of 10 and 20 ppm, always significantly (.05 level) less sensitive than family 6-13 x 2-8.

<sup>1</sup> 9/3/76 and 10/14/76 values were collected on secondary needles and are averages of two replications.

<sup>2</sup> Values in any column followed by the same letter are not different at the .05 level.

<sup>3</sup> Number of families that exhibited no foliar symptoms.

<sup>4</sup> Probability of a greater F value occurring by chance resulting from ANOVA test of differences among families.

<sup>5</sup> Number of weeks since 1-year-old trees broke bud and developed secondary needles.

TABLE 2. Average tree symptom severity index (SSI) for individual trees of loblolly pine families exposed to 20 pphm O<sub>3</sub> on 7/14/75 and then to 25 pphm O<sub>3</sub> on 9/3/76. Family 6-13x2-8 and family 540 x 504 were most consistent in being most sensitive and very tolerant, respectively

Family	Tree	7/14/75	9/3/76
		20 pphm	25 pphm
6-13 x 2-8	1	10	46
	2	2	43
	3	29	173
	4	4	14
2-40 x 2-8	1	7	16
	2	17	8
	3	29	21
	4	11	18
546 x 523	1	0	4
	2	2	15
	3	2	43
	4	0	10
6-7 x 508	1	1	13
	2	3	7
	3	4	5
	4	20	2
531 x 517	1	0	1
	2	27	0
	3	10	1
	4	27	18
526 x 517	1	5	4
	2	1	29
	3	1	66
	4	6	8
540 x 504	1	0	0
	2	2	0
	3	0	0
	4	0	0

<sup>a</sup>7 of 18 families exposed were selected to illustrate that within some families the trees responded similarly relative to each other from year to year, while in other families they did not.

TABLE 3. Average tree symptom severity index (SSI) of selected loblolly pine families over a variety of exposure conditions at different times of the year. Tree age was held relatively constant for each treatment

Exposure Date 1975	pphm O <sub>3</sub> /hr	Symptom Severity Index			
		6-13 x 2-8 <sup>a</sup>	540 x 504	Most Sensitive	Most Tolerant
4/22	50/8	34	0	34	0
5/5	25/8	---	0	8	0
6/9	25/8	---	0	1	0
7/2	15/8	175	1	175	0
7/2	25/8	245	64	245	1
7/8	15/8	299	0	299	0
7/8	25/8	376	32	376	1
7/14	20/8	11	0	59	0
7/22	20/8	1	0	8	0

<sup>a</sup> 6-13 x 2-8 - selected as a sensitive family at the conclusion of the screening studies.

540 x 504 - selected as a tolerant family at the conclusion of the screening studies.

Most sensitive - the family exhibiting the greatest foliar damage for a particular replication.

Most tolerant - the family exhibiting the least foliar damage for a particular replication.

Part of the usefulness of white pine as a bioindicator had been the variability among clones in response to different pollutants (2, 3, 11, 13). One clone might be very sensitive to O<sub>3</sub>, but not to SO<sub>2</sub>. Another might be sensitive to SO<sub>2</sub>, but not O<sub>3</sub>. Therefore, the bioindicator system with white pine may be useful in determining what kind as well as how much pollution is present. Some indication that this may also be true with loblolly pine was found in this study. Family 4-5x523 was not particularly sensitive to O<sub>3</sub>, but was the most sensitive to NO<sub>2</sub>. Family 6-13x2-8 was sensitive to both pollutants. Additional research has shown that 4-5x523 was sensitive to O<sub>3</sub>, NO<sub>2</sub>, and SO<sub>2</sub>, while the reciprocal (523x4-5) was sensitive to O<sub>3</sub> and SO<sub>2</sub>, but much less sensitive to NO<sub>2</sub> (9). This type of response, if it can be developed and quantified, would add immeasurably to the value of a bioindicator system employing loblolly pine.

The time of the year effect was an interesting but puzzling problem. It has been noted by other researchers on several other species of plants (5, 7, 8, 10). Peak sensitivity, holding age constant, usually occurred at the end of June and the first part of July. It may have been tied partly to age, day length, and RH in a field situation, but even controlling those variables the difference still occurred. The dramatic difference noted in this study may be due in part to RH. During the winter months and spring months, it was difficult to maintain high RH levels in the exposure chambers. This effect may be very significant in the field, especially near point sources. At times when plants are not in a sensitive condition, the pollutant levels could possibly be higher without causing significant adverse effects to the plants.

The results indicate that sensitivity to O<sub>3</sub> may be heritable, and warrant further study into the feasibility of selection or breeding programs. Among the 18 families tested, two involved the parent tree 2-8 and three involved the parent tree 504. Families with 2-8 were usually more sensitive than families with 504. In overall ranking of the 18 families, the two most sensitive involved 2-8 and three of the four most tolerant involved 504. The parent trees have never been placed under experimental pollution stress, so the parent responses are unknown.

Weir (15) predicted genetic gains of 26 to 32 percent (later adjusted downward to 20 to 26%) for mass selection among trees in the north coastal population (North Carolina). In his summary, he stated that the potential for achieving greater genetic tolerance depended on whether the response to acute ozone doses (which he used) was representative of the responses to ambient levels, and whether there was an economically important growth reduction associated with low ozone tolerance. In the absence of such information he concluded that selection and breeding were not economically feasible at this time.

The current study has demonstrated significant differences in foliar response between families at pollution levels closer to ambient. Further work has demonstrated significant differences in foliar symptoms and significant differences in height growth of two families selected from this

study in response to concentrations of O<sub>3</sub>, NO<sub>2</sub>, and/or SO<sub>2</sub> below the National Ambient Air Quality Standards. Additional work with open-top chambers has demonstrated up to a 40% reduction in height growth of an O<sub>3</sub>-sensitive family in response to ambient air pollution in the absence of foliar symptoms. Dorman (4) has stated that "Breeding for resistance to minor pests may not be justified at this time because more important traits for yield have higher priority, but it would be wise to insure that breeding does not increase susceptibility to minor pests. Should this occur, losses to minor pests could offset some of the gains from improvement of other traits." In the opinion of the authors air pollution is more than a minor pest. The authors have reported up to a 40% height growth reduction due to ambient levels of air pollution and Phillips et al. (12) have estimated 45% radial increment growth reduction in loblolly pine. However, even if air pollution is to be considered as a minor pest, tree improvement scientists should be cautious about selecting unknowingly for sensitivity to air pollution.

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DISEASE CONTROL - THE MODERN WAY

Kenelm Russell<sup>1</sup>

Mike Srago<sup>2</sup>

A short dialog exposing the intricacies of FIFRA and other acronyms.

CHARACTERS: Phellinus P. Weirii, "Phel" portrayed by Ken Russell, a hardnosed pathologist employed by the State Department of Natural Resources, Protector of Water, Air, Soil and General Overseer.

Phomopsis Smith, "Phome" portrayed by Mike Srago a frustrated self-educated nurseryman employed by the Cut & Scram Timber Company, locally known as C&S.

SCENE: Our discussion opens in Phel's lab where Phome has stopped to find out how he can control the epidemic of droopy twig on his Douglas-fir seedlings caused by the fungus Basidioascus twospore.

Phel: Well, Phomopsis Smith, Hello there! How does your garden grow today?

Phome: Oh man, do I ever have a problem. I've got 23.6 million 1-0 Douglas-fir seedlings that will probably die by Monday if I don't spray them by the end of the week. They look terrible.

Phel: Did you bring a sample?

Phome: Yeah, here.

Phel: (Peering under microscope) Hmmmmm! They've got the gascutis (A T. Childs concoction) all right! It's droopy twig, caused by the fungus Basidioascus twospore. Here Phome, take a look (Phome peers under the scope, too).

Phome: Oh, I gotta spray tomorrow for sure.

Phel: Well, wait a second. What are you going to use?

Phome: I bought this methyl perocidetyl alkaline bichloride fungicide commonly called Zit. I'm going to use that. The salesman said it was good for everything from mule's ear to wind suck. He said it even controls fungus diseases, too! I read the label, but I didn't see droopy twig on it anywhere, but that's OK.

The label says to use Zit at 12 lbs/100 gallons of water for mule's ear so I'm going to increase the rate to 48 lbs. of Zit for droopy twig just to be sure of good control.

Phel: Whoa - wait a minute Phome. That's a bunch of Zit! The regulations won't allow you to do that.

Phome: Regulations, what regulations? I read the label! Isn't that enough? It says in big letters DO NOT APPLY OVER WATER. Since I'm going to apply the stuff with a helicopter, I'll be real careful. I'll instruct the pilot to go over our creek extra fast. That way only a small amount will go into the creek. Besides, it'll wash downstream after a few hours. Won't bother us any.

Phel: Uh - Phome? Have you got a little time? I'd like to talk to you about the new approach to controlling your disease. We must follow the procedure.

Phome: Sure, we've got to look out for our environment now, but don't forget about my droopy twig.

Phel: Do you have an operator's license to use Zit?

Phome: No, who needs that? We're so far out in the brush that the bartender is a sasquatch.

Phel: Well, you must have an operator's license to use Zit in your operation. There are some other problems in its use, but we'll cover that in a minute.

There is a course going on now at the Department of Natural Resources, Protector of Air, Water, Soil and General Overseer's office right now. I want you to attend. You'll have to study, and there will be a pop quiz at the end to certify that you know your stuff. If you pass the test, the State Dept. of Agriculture will grant you your operators license. You'll learn about the Federal Law governing fungicides, and how it affects your way of doing business. Further, you'll see how the State functions in the process, too. You'll also find out that there are several different kinds of pesticide licenses. Mine, for example, is a Public Consultant's license. I cannot prescribe for anything but fungicides because that is what is stamped on my license. I could be held liable if I strayed off course and something went wrong with my recommendation.

Phome: Wow, what's this world coming to?

Phel: Somebody's always looking after you, Phome. By the way did you ever hear of FIFRA?

Phome: Who's that? Your latest girl?

Phel: No, no, that's a Federal Act designed to protect the "user" and the "usee" against accidents with pesticides. It also provides many other officially sanctioned methods of dealing with fungicides, insecticides and rodenticides.

Phome: Only Act I'm familiar with is the Sex Act, and it generally doesn't provide against any accidents.

Phel: Uh, yeah, well let me tell you about FIFRA and how it will affect our treatment of your droopy twig. Let's start with the Feds and work our way to the State.

(Description of Laws on pesticides follows.)

Let's go back a few years. The first Federal Insecticide Act was passed in 1910. It provided protection for farmers against adulterated or misbranded products. It was buyer beware before that.

In 1947 the first Federal Insecticide, Fungicide and Rodenticide Act was passed. That's FIFRA, Phome, not my girl. The Act placed the burden of proof of acceptability of a pesticide on the manufacturer prior to marketing it. The user, consumer, and the public would then have protection against potentially dangerous pesticides.

A year later, minimum tolerances were established for pesticides. Their registration was on the basis that either no residue remained or that residues were declared safe.

In 1961, more protection clauses were written in for fish, wildlife and environment. Eventually, concern built over the continued use of persistent pesticides. This concern probably helped push development of the newer law. With the advent of EPA's establishment in 1970 the modern day FIFRA evolved. The 1972 act required all pesticides be registered and tolerances hazardous to environment, flora, fauna, etc., be accounted for and established. The act itself is only 22 pages and 27 sections long, Phome, but there are hundreds of pages of regulations and guidelines that dictate specific enforcement procedures.

Phome: It's not as long as this script!

Phel: In 1975, additional amendments to FIFRA were signed into law. The amendments were mainly for clarification of original congressional intent.

I'll skim through those sections briefly for you Phome, to put the whole thing in perspective.

Section 3. Registration of Pesticides - All pesticides must be registered with EPA. Some were previously covered by State authority only. There is an exemption clause under an experi-

mental use permit. This section tells how data to support registration is handled, denial procedures and classification (general vs. restricted) of pesticides.

Section 4. Restricted Use Pesticides, Certified Applicators - Under this section the lawmakers recognized the need for separation of highly toxic from more general pesticides, Phome. They call the toxic ones Restricted Use Pesticides. You must be certified and highly trained to use them. States handle the training and individual State programs must follow prescribed standards which are dictated by the EPA Administrator. We'll talk more on training later.

Another important part of this section is to make sure the States provide Integrated Pest Management techniques in applicator certification programs. Training is done under section 23(c) of the Act.

Section 5. Experimental Use Permits - This section allows experimentation and development of new products. It includes the setting of temporary tolerance levels if they are needed and adherence to details in the previous section. Public or private agricultural research agencies, laboratories and universities may secure an experimental use permit provided the pesticide under investigation is used for experimentation. I have used these permits several times, Phome. In one Christmas tree disease application the State Dept. of Agriculture issued my permit restricting me to under 10 acres for treatment. They required a letter from me identifying the pest, pesticide to be used, proposed treatment and spray schedule. We could spray your trees under this Section, Phome. One time, I was able to get a State experimental use permit with only a three day notice. Federal permits for a restricted use fungicide would take much longer. Let's come back to this Section later, Phome, to see how we handle your special need.

Section 6. Administrative Review, Suspension - Registration of a pesticide is cancelled 5 years following date of registration or any five year period thereafter, unless the registrant requests continuance. Things aren't permanent anymore Phome, and that's for the good!

Sections 7, 8, 9 & 10 deal with Registration of Establishments, Books and Records, Inspection of Establishments, etc., and Protection of Trade Secrets. These rules have more to do with the Pesticide manufacturers than us, Phome.

Phome: Has a lot to do with me if they require inspection of "Establishments".

Phel: Uh, No Phome, they're not referring to that kind of "Establishment".

Phome: Oh!

Phel: Section 11 deals with Standards Applicable to Pesticide Applicators. This requires commercial operators to have separate standards from private applicators. For instance, if you were a commercial applicator, Phome, you would have to keep records on pesticides such as date, application rate and other data.

Section 12. Unlawful Acts contains the "no, no's". This covers things like repackaging, non-tasty coloring or issuing restricted use pesticides for an untrained user, plus many others. A classic example is the time I was called out to look at some mysteriously dying western hemlock in a back yard. Under the hemlock were some fenced in pigs who had done a lot of soil damage. Everytime I tried to diagnose the cause I kept coming back to the pigs. I just knew the pigs couldn't have killed the trees because there was no direct damage.

Finally, in desperation, I asked the lady, with the problem, if anything had been applied to the trees or near them. After some hesitation she said "Well, her husband had brought home some white stuff in a can from work which was given to him by one of his buddies who said it really worked. He used it in the back yard to kill salal." (The hemlock trees were about fifty feet from the salal.) Now we are getting somewhere, I thought, and I felt the pigs were off the hook. Her husband had sprinkled the white pellets liberally around the salal, which was readily killed and the winter rains later washed the dissolved pellets into nearby trees downslope. The substance was picloram, a very potent and persistent brush killer to be used only by highly trained personnel. Once the nearby trees picked up lethal doses, the chemical was transferred by "flashback" to other trees nearby. Nearly the whole stand was killed. A sad case through careless use of white pellets in untrained hands from an unlabeled coffee can, all illegal of course.

Section 13 covers Stop Sale, Use, Removal and Seizure.

Section 14 covers Penalties. It could cost you money, Phome, in the form of a fine (up to \$1000) if you applied your Zit without a license or its proper registration.

Section 15. Indemnities. This section helps pay for pesticides a distributor might be stuck with if they are found hazardous and pulled off the market.

Section 16. Administrative Procedures - Judicial Review is concerned with all the legal stuff such as hearings, time periods, etc., necessary for any change in registration status of a pesticide.

Section 17. Imports and Exports - The only requirement under FIFRA for export is that Section 8 Records, be followed. All other adherences must be requirements by the purchasing foreign country. Foreign governments are notified of cancellations of pesticides through our State Department. The 1972 law is a little weak here.

Section 18. Exemption of Federal Agencies - Your droopy twig problem could come under this section Phome, but I'm afraid the problem isn't big enough or a real emergency on a large regional scale. For example, a "Section 18" was done on the 1974 Douglas fir tussock moth problem in Washington and Oregon when DDT was authorized through the EIS process. Also, there is a considerable time constraint using Section 18 which would cramp your style, Phome. We would have to begin detailed preparation up to six or more months prior to spray. No Monday morning spray, Phome following a Tuesday previous awareness of a problem.

Phome: What can I do?

Phel: Hold on just a bit and we'll get to that.

Phome: My trees might die before you get there!

Phel: Section 19. Disposal and Transportation - Establishes procedures for storage, disposal and transportation of packages and containers of pesticides. They are very strict.

Phome: Does that mean I can't toss my old pesticide cans out by the creek anymore? The water takes all the material away and it doesn't bother us at all.

Phel: I didn't even hear that, Phome, uh, lets go on.

Section 20. Research and Monitoring - Explores research to develop alternatives to chemicals such as biologically oriented control. It's usually done under contract to universities or other agricultural research organizations. Likewise, monitoring of air, soil, water, man, plants, and animals for adverse effects from pesticides is done.

Section 21. Solicitation of Comment, Notice of Public Hearings - Before regulations are published under FIFRA the views of the Secretary of Agriculture must be notified. This is where scientists, farmers, farm organizations and other qualified persons provide input before regulations are published. Notices are published in the Federal Register in timely fashion.

Section 22-24. These are the most meaningful sections of the FIFRA act for us, Phome. Here's where a great deal of authority transfers to the State. Section 22. Delegation and Cooperation - Delegates authority to the State.

Section 23. State Cooperation, Aid, and Training dictates that EPA enters cooperative agreements with States for enforcement of regulations of FIFRA. The States provide the training needed to qualify pesticide applicators and consultants. The help of the Cooperative Extension Service is also enlisted to assist in training of farmers of accepted uses. This is where our licenses come from, Phome.

Phome: That all sounds complex at first, but now I'm beginning to see why. I'll be their star pupil at the training session. But what about my droopy twig? I still don't have a solution.

Phel: Coming right up Phome.

Section 24. Authority of States Part (c) - Provides a lot of help and is probably one of the most used sections of FIFRA. Remember 24(c). It can bail us out under many circumstances.

In essence, the States regulate the use of the pesticides while working through the FIFRA law and using its regulations. Section 24(c) leaves us with a possibility with your droopy twig, Phome. It says that the State may grant a supplemental label for a Special Local Need (SLN) but the recommendation must come from a recognized disease specialist or researcher. "Hak Koff", that's me, Phome.

Sometimes research may not be required. There is one important fact to remember though. Someone has to accept liability for the special labeling. The manufacturer almost always does this, but it's often up to people like us to point out the need. Usually, agencies don't want the liability imposed by the registration. Occasionally, an agency or company may accept the liability but the pesticide manufacturer must agree to it. I have an example or two I can show you.

Remember the 24(c) provision and the SLN label and also remember that the chemical companies are skilled at processing them. In extreme emergencies they can be processed quite rapidly - sometimes in as little as a few days.

By comparison, a Federal permit could take 5 or 6 years. The SLN label must also tie back to pathogen by species and the host.

Complete health risk analysis must be done if the compound to be used is highly toxic. Usually, 24(c)'s are issued for fungicides that have had considerable testing and long use on other pathogens; their safety hazard is low or average and the label is for an additional disease it has not been tested on.

Well, we've just about covered the FIFRA law except for the last three sections; 25 - Authority of Administration, 26 - Severability and 27 - Authorization for Appropriations. Section 25 describes the procedure for preparing the regulations including the required timetables. There is also provision for a Scientific Advisory Panel that reviews and comments on impacts of pesticides on health and environment following proposed action under the regulation time table.

Now lets deal with your poor Douglas-fir seedlings. I've done some work with Zit on diseases similar to droopy twig, Phome. It is a registered fungicide now. I suspect it will work on preventing the disease on more of your seedlings. We must do something quickly. Remember, we work through the State on this. I'll write a letter to the State Dept. of Agriculture and will phone at the same time. They will probably issue an experimental use permit based on my experience with the fungicide. We could have the permit through the State Dept. of Agriculture by next Tuesday. We will make three applications of Zit at the standard package recommended 12#/100 gallon rate plus a good spreader-sticker and carefully observe our results.

We must leave a small untreated patch as a control. Our results under this test will then form the basis for a 24(c) label application we can apply for next spring, provided our treatment is successful. If everything works we won't have any difficulty. Uh, by the way, you'll have to be licensed to spray so we'll have to find a certified operator to do it the first time.

Phome: Aww-

Phel: Congress and EPA have been working on a new FIFRA for many months and it was signed into law by President Carter on September 30, 1978. The new Act won't be printed for awhile but the Senate Conference Report (95-1188) gives details of all of the changes made to FIFRA.

Lets take a look at the changes because they make FIFRA a much easier law to work with.

#### STATE AUTHORITY

Most significant in the new Act is the enhanced authority of the States over the program.

First, there are expanded conditions for additional uses of federally registered pesticides which may be issued under 24(c) by the States. Changes will allow the State to better meet special local needs if registration for such use has

not been previously denied or cancelled by EPA. Related to this, is the minor use issue which has been a problem with FIFRA ever since the 1972 amendments. It concerns high value crops requiring small amounts of pesticide.

A typical example is the southern forest nursery industry with 1,300 acres of seedlings valued at \$13 million. It costs only \$75/acre to control weeds with herbicides, but without them weeding costs would be \$700/acre. Pesticide manufacturers are reluctant to consider the small user, because of low profit potential. The new Act resolves this critical problem.

EPA is required to make standards for minor uses commensurate with anticipated extent of use, pattern of use and degree of potential exposure from the pesticide being considered to humans and environment. In the development of the standard, EPA is to consider economic factors of potential national volume, distribution and impact of cost of meeting all requirements. Thus, incentive for producing minor use pesticides will not be destroyed.

Second, the States also will have important new enforcement authority. Those States with cooperative enforcement agreements will have primary enforcement responsibility under the Act.

Third, Congress will provide each State or Indian tribe up to 50% of the anticipated cost for conducting applicator training and certification programs.

#### REGISTRATION AND REREGISTRATION

There are other improvements under the complex registration process. The old process has stalled registration and reregistration of some 40,000 pesticides. A Conditional Registration amendment has been provided in certain situations to greatly alleviate the congestion even though data collection required for registration or reregistration is not complete. Safeguards are included to protect the public interest. This authority will be exercised on a case-by-case basis. It should avoid leaving certain crops in a no man's land when a serious pest threatens and alternatives are not available.

Second, EPA can now classify for Restricted or General Use currently registered pesticides without waiting for the drawn out process of registration. This makes it easier to implement existing law requirements which limit use of restricted pesticides to certified applicators.

Third, the revision provides relief in the controversy manufacturers have whose test data is used by competitors for

registration of their data. There is now compensation protection of all test data for 15 years. There are additional protection amendments for companies which Representative Tom Foley (Washington) says are among the most important refinements to FIFRA. They will promote safer and more effective pesticides and stimulate a flow of new research providing new basic ingredients.

Fourth, there is also relief from the RPAR process. Thank heavens! Phome, have you ever heard of this process?

Phome: Yeah, I did that just after I got up this morning.

Phel: No, not that! RPAR stands for Rebuttable Presumption Against Registration. RPAR's are initiated for a given pesticide when certain criteria in the FIFRA regulations are exceeded. These include human health hazards and unacceptable environmental impact. When a pesticide makes the RPAR list it means the manufacturer must show cause or "rebut" why the pesticide should be registered. RPAR's may be initiated against either currently registered pesticides or a new one.

Under the amended FIFRA, pesticides will not be subjected to RPAR until substantial evidence is presented raising concern of adverse risk to man or environment. Notice of such evidence will be published in the Federal Register.

In order to minimize the process, EPA will develop scientific protocol for the development of human exposure data. EPA will then work with the registrant to assemble, collect, evaluate and weigh data prior to initiating RPAR. A risk-benefit evaluation will be made. If humans or environment are not at risk or exposure is minimal RPAR will be forgone and registration will continue. This is quite an improvement, as it will now take significant evidence of hazard before initiating RPAR.

#### COMMERCIAL APPLICATORS

New penalties for violation of FIFRA by commercial applicators while using pesticides will be in force. First offenses will no longer be just a warning, as under current law, but will hit the pocket book in the form of fines of varying amounts depending on the infraction.

There are also some changes with regard to inspection of books and records. Owners must be notified in writing including a statement as to whether a law violation is suspected.

## MISCELLANEOUS

Other changes include provisions relating to use of a registered pesticide in a manner inconsistent with its labeling, the use of a pesticide at a stronger concentration than that on the label, requirement for a study on pesticide application methods, arbitration procedures to resolve disputes on data requirements, waiver of data requirements pertaining to efficacy, changes in classification from restricted to general use, changes in effective dates for certain required actions and provisions relating to experimental use permits and pesticide research.

No doubt of considerable interest to Canadian friends are the new conditions imposed on pesticides and devices intended for export. These conditions will allow foreign countries to be better informed concerning US manufactured pesticides.

In addition, FIFRA has a built in Sunset Provision. Each year EPA must seek an extension and justify to Congress activities it has taken during the intervening period. In this manner, problems that arise during the year can be resolved through an oversight process.

One of the new FIFRA sections (28) IDENTIFICATION OF PESTS; COOPERATION WITH DEPT. OF AGRICULTURE'S PROGRAM will undoubtedly filter down to many of us scientists and specialists rather soon. This section states that all pests that must be brought under control are to be identified. Some of this has been done all ready for the Pacific Northwest, at least in broad categories. EPA and USDA must coordinate research and implementation programs to develop and improve safe use and effectiveness of chemical, biological, and alternative methods to combat and control pests that reduce the quality, economical production and distribution of agricultural products to domestic and foreign consumers.

Rep. Foley feels the amended act will balance in the direction of providing necessary chemicals and herbicides needed for production of food and fiber with an eye on environmental safety. All who worked on the amendments from both sides openly tried to remove inhibitions and problems and develop a more agriculturally oriented program than in the past. Congress really went to bat on this law, Phome. In the words of Rep. Thone (Neb.) and echoed by several others; they want EPA held on a tight rein. Congress is very concerned about law that is written by bureaucrats. They really did something about it in the FIFRA amendments.

Phome: That's it, huh!

Phel: Yup! The new law is now 31 sections long and a few more pages than before, but it will reduce a lot of problem areas.

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NOTES ON THE BIOLOGY AND CONTROL OF SOME JUNIPER AND  
PINE DISEASES IN THE GREAT PLAINS

Glenn W. Peterson<sup>1/</sup>

DOTHISTROMA BLIGHT OF PINES

Dothistroma pini which causes a blighting and early casting of needles of pine species such as ponderosa, Austrian and mugo can be effectively controlled by properly timed applications of copper fungicides. In eastern Nebraska, infection of second-year and older needles may occur as early as late May (8). First-year (current-year) needles of Austrian and ponderosa pines are initially resistant to infection; they become susceptible near mid-July (3). Thus two applications of fungicide will provide complete control. An application made in mid-May will protect older foliage; a second application in mid-June will protect the first-year needles (2, 3, 9). The extent of early infection (prior to mid-June) is low in most years; hence good control can usually be obtained with one spray applied in mid-June.

An interesting phenomenon has been observed during the germination of D. pini conidia. Plastic prints of the surface of needles collected from Austrian and ponderosa pines in the field revealed that when conidia of D. pini germinate, their germ tubes are positively directed toward stomates (5, 14, 16, 18). Since the fungus enters needles through stomates, the positively-directed germ tubes increase the chances of infection over germ tubes that grow at random.

Interestingly, when artificially inoculated pines are incubated at 100% relative humidity, germ tubes of D. pini are seldom directed toward stomates. A moisture gradient from stomates to germ tubes is likely responsible for the positively directed growth of germ tubes in the field, but incubation at 100% relative humidity destroys the gradient. Thus chances of infection are less when inoculated plants are incubated at 100% relative humidity. This phenomenon is probably more widespread than has been realized previously, but artificial incubation at high relative humidity has not revealed this. Conidia of Scirrhia acicola also have positively directed germ tubes in the field (16). Tests are being made to see if this phenomenon occurs in other host-fungus systems in which the fungus enters through stomates. This phenomenon would be particularly significant in forestry research involving mass inoculation of trees for selection of resistant individuals (i.e. white pine blister rust, brown spot).

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## DIPLODIA BLIGHT OF PINES

Diplodia pinea infects a large number of pine species, including Austrian, ponderosa, Scots, mugo, and red pines. The fungus has most frequently been reported on Austrian pine; furthermore, damage has seldom been reported in other than plantings of pines. Damage is usually not severe in plantings in the Plains until trees are over 30 years old (4). Severe infection of older trees may be related to the buildup of inoculum on seed cones. Second-year seed cones of Austrian, ponderosa, and Scots pines are highly susceptible. Infection of plants younger than 30 years has been noted, including seedlings in nursery beds, but this has usually been observed when the younger trees are near old infected trees.

Infection of new shoots of Austrian, ponderosa, and Scots pines occurs, as soon as buds start to expand. Infection on nonwounded new shoots can occur beginning with opening of buds and extending to about mid-June (1). The period of high susceptibility, however, is a two-week period beginning with bud expansion (12). In eastern Nebraska, this period is from about April 24 to May 8. Two applications of Bordeaux mixture during this two-week period has effectively reduced the amount of shoot infection (12, 13).

## PHOMOPSIS BLIGHT OF JUNIPERS

The most damaging disease of conifers in Plains nurseries is caused by the fungus, Phomopsis juniperovora. Under epidemic conditions, entire beds of Juniperus virginiana and J. scopulorum can be destroyed if chemical control measures are not used.

Phomopsis initially affects foliage, then spreads to and kills stem tissues. Newly developing needles are especially susceptible while they are in the yellowish-green stage; after needles develop a normal deep green they are not susceptible (7). When a side shoot is infected, the fungus progresses to the main stem, which it may girdle if the stem is less than 1/3 inch in diameter.

Established junipers in plantings can be infected; but since only small diameter branches are girdled, these established junipers are not killed even when infection is severe. Thus Phomopsis blight is primarily a nursery problem.

Phomopsis can be effectively controlled by mercury fungicides, (15); however, now that there are restrictions on mercury fungicides, a search has been made to find other effective fungicides (6).

Studies by Dr. J. Otta have shown that Benlate applied at the rate of 0.5 lb/acre every 7 to 10 days to J. virginiana seedlings coupled with weekly roguing of all seedlings with dying foliage has given complete control of Phomopsis blight at the Big Sioux State Conifer Nursery, Watertown, South Dakota.

## CERCOSPORA BLIGHT OF JUNIPERS

The fungus, Cercospora sequoiae var. juniperi infects a number of Juniperus species and other species in the Cupressaceae. Well established Juniperus virginiana and J. scopulorum, have been killed in plantings in the central Great Plains (17). Plantings of J. monosperma in western Iowa have been destroyed by this fungus. The disease progresses more rapidly on J. scopulorum than on J. virginiana in the Plains (11).

Infection typically occurs first on foliage of lower branches near the stem and progresses outward and upward. Infected branches often have only a tuft of healthy foliage remaining on their tips.

On J. virginiana and J. scopulorum, both current and previous years' juvenile leaves become infected, whereas only the previous years' spur (adult) leaves become infected (10). Initial infection occurs as early as 21 June in eastern Nebraska. Symptoms have been observed in July, but symptom development is usually not striking until late September (10).

A highly persistent fungicide applied before late June theoretically could protect trees with spur foliage for the entire season. Because of weathering (Bordeaux mixture), an additional application may be required.

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## BUILDUP AND IMPACT OF ELYTRODERMA DEFORMANS

### ON JEFFREY PINES IN CALIFORNIA

R. F. Scharpf

R. V. Bega

The fungus Elytroderma deformans (Weir) Darker is responsible for the most serious needle disease attacking ponderosa pine (Pinus ponderosa Laws), Jeffrey pine (P. Jeffreyi Grev. and Balf.), lodgepole pine (P. contorta Dougl.) and several other pines in western North America. Weir (1916) first recognized and reported the importance of this disease agent. Since then, it has received substantial attention and research by some of the best known forest pathologists, including Childs, Lightle, Roth, Wagener, and Waters. Much information about the biology, distribution, hosts, and damage of the disease is known but details about its epidemiology, the damage it causes different forest stands, and control are less well known. For reasons not well understood to us, relatively little research has been conducted on this serious forest disease within the past decade.

This report points out why Elytroderma disease is considered a serious disease of western conifers and why its presence should be considered in the management of certain forest stands. Results presented here were obtained from studies of the disease in Jeffrey pine stands near South Lake Tahoe, California, with a known history of Elytroderma infestation.

The disease has been known in these stands for at least 40 years and at least one outbreak took place in 1949-1950 (Wagener, 1951). No other outbreaks were recorded until Miller (1970) reported buildup of the disease in the South Lake Tahoe area in 1970.

The objectives of the study were to: 1) determine the incidence, rate of spread, and buildup of the disease in Jeffrey pine stands following an "epidemic" outbreak of the disease; and 2) determine the effect of the disease on mortality, growth, and vigor of Jeffrey pines. The study was begun in 1971--the year of the outbreak--and continued until fall 1977.

#### METHODS

Six plots, each containing about 100 test trees, were established in or near the area of disease outbreak. Plots ranged in size from about 0.2 acre to slightly over 1 acre. Basal areas ranged from 53 square feet to about 225 square feet per acre among the plots. More than 95 percent of the basal area on all plots was in Jeffrey pines. Some plots were in areas of light infection and others in areas of heavy infection. All plots were within a few hundred yards of one another so that differences in topography, stand composition, and microclimatic

conditions could be held to a minimum. The plots consisted mostly of Jeffrey pines but a few trees of other species, such as white fir, Abies concolor Gord. and Glend., and lodgepole pine, were also present.

Tree measurements and tree condition were recorded at the beginning of the study. Included were diameter, tree height, crown class, and tree vigor. Disease distribution and intensity was also recorded. Disease distribution was determined by visually dividing the living crown into four parts--lower, lower-mid, upper-mid, and upper crown--and then determining the presence or absence of the disease in each portion. A total of the parts of the crown infected was used as a measure of disease distribution within the tree.

Disease intensity was based on an estimate of the total amount of foliage showing disease symptoms within the living crown: light infection-- 1 to 10 percent of the foliage showing symptoms, moderate infection-- 11-50 percent foliage with symptoms, and heavy infection -- more than 50 percent of the foliage showing symptoms.

Permanent photo points were established and color photos taken of certain trees each year from 1971 to 1977. Tree mortality was recorded once, sometimes twice, a year during this period. At intervals of 2 and 5 years, the trees were remeasured, their condition noted, and disease distribution and intensity recorded.

## RESULTS

### A. Host condition and disease situation in June 1971.

Test trees ranged from 1 to 40 inches dbh with an average dbh of 8.9 inches. Crown and vigor class of the test trees is summarized below.

<u>Crown Class</u>			<u>Vigor Class</u>		
Dominant (percent)	Co-dominant intermediate (percent)	Suppressed (percent)	Good (percent)	Average (percent)	Poor (percent)
43	37	20	29	40	31

Distribution and intensity of the disease was also recorded at the beginning of the study:

<u>Disease Distribution</u>					<u>Disease Intensity</u>			
(percent live crown affected)					None	Light	Med.	Heavy
None	1-25	26-50	51-75	76-100	(percent of test trees)			
0	1	1	22	76	0	28	25	47

None of the test trees was completely free of infection by Elythroderma, and nearly all trees were infected throughout most of their crown. About half of the test trees were rated heavily infected and half light to moderately infected. Infection did not appear to vary noticeably among trees of different size or crown class.

Intensity of infection did vary among trees of different vigor class:

<u>Vigor Class</u>	<u>Infection Rating</u>		
	Light (percent)	Moderate (percent)	Heavy (percent)
Good	61	28	11
Average	23	34	43
Poor	5	13	82

In general, trees of good vigor were lightly to moderately infected; whereas trees of poor vigor were heavily infected. It appeared that trees were of poor vigor because of the disease rather than poor vigor trees being more subject to attack by the disease.

B. Changes in host condition and disease situation from June 1971 to August 1977.

#### 1. Tree mortality

Mortality was heavy among the test trees over the six-year period of study:

<u>Month/Year</u>	<u>Living Trees</u>	<u>Dead Trees</u>	
		No.	% of living trees
June 1971	607	--	--
June 1972	581	26	4.3
June 1973	553	28	4.8
June 1974	522	31	5.6
June 1976	447	75 <sup>1/</sup>	14.4
Aug. 1977	439	8	1.8
<hr/>			
Total	607	168	27.7
<hr/>			
1/ 2 year mortality			

Over the six-year period of study, more than one-fourth of the test trees died. Of the trees that died, 90 percent were heavily infected with disease in 1971. In addition, 72 percent of the mortality occurred among trees of poor vigor and 25 percent among trees of moderate vigor. Few trees of good vigor in 1971 died during the six-year period.

Mortality was fairly evenly divided among trees of different crown class. Among the dead trees recorded, 33 percent were dominant, 40 percent co-dominant and intermediate, and 27 percent suppressed.

A biological evaluation of the infested area in 1973 showed that agents other than *Elytroderma* also killed Jeffrey pines (Pierce and Srago, 1973). Causes of mortality were recorded as *Elytroderma* disease alone, *Elytroderma* disease and Jeffrey pine-beetle, Jeffrey pine-beetle alone and unknown causes. We found on our study plots, however, that nearly all the mortality could be attributed to *Elytroderma* disease alone or to the interaction of *Elytroderma* disease and Jeffrey pine-beetle.

The severe drought of 1976-1977 did not appear to have increased mortality in our study area as it did in many other areas throughout the state.

## 2. Tree vigor and disease rating changes

Changes in tree condition and in disease rating were determined for the test trees in 1973--2 years after the study began and after 6 years, in 1977. For this report, emphasis will be placed on the 1977 examination.

Of the trees that were still alive 6 years later, about half showed no change in disease intensity rating, about a third increased in disease intensity, and the rest decreased in disease intensity. These results in 1977 were nearly the same as those found in 1973, indicating that there was no noticeable building of disease in test trees over the six-year period of study after the initial outbreak of *Elytroderma* in 1971.

Another fact that suggests lack of disease spread or building in test trees since 1971 was that the disease was less widely distributed in the crowns of trees in 1977. For example, crowns of nearly 90% of the test trees showed about the same amount of infestation, or less of it, in 1977 as in 1971. Newly developed crown and top growth were free

of Elytroderma. As a result, the disease was present in a smaller proportion of the living crown of most trees in 1977 than it was in 1971.

Host vigor declined dramatically among test trees over the six-year period. More than 50 percent of the trees declined at least one vigor rating, and almost all the others remained the same. Few trees improved in vigor. Thus, for the stand as a whole, not only has the stocking level dropped as a result of mortality after 6 years, but the average vigor of the surviving trees has also declined markedly.

Results on the effects of the disease on radial growth and stand basal area over the 6 years of study are not yet available. But preliminary analyses indicated that both radial growth and basal area were reduced markedly in heavily infested stands.

#### DISCUSSION AND CONCLUSIONS

Serious mortality of Jeffrey pines resulted over a six-year period from this epidemic outbreak of Elytroderma needle disease. Weakening of trees as a result of the persistence of the disease in the stand after the outbreak, along with Jeffrey pine-beetle activity accounted for most of the mortality. Thus, single outbreaks of the disease in certain stands can result in severe persistent infestation, loss of tree vigor, and subsequent mortality associated with both the disease and bark beetle activity. Some of the factors related to mortality were the same as those reported by Childs (1968) for Elytroderma disease on ponderosa pine in the Northwest: 1) Mortality occurred among trees of all size classes; 2) mortality was highest among trees of poor vigor; 3) mortality was the result of the association of Elytroderma disease with other agents.

We noticed, as did Childs and others (1971) and Hunt and Childs (1957), that outbreaks of the disease are restricted to certain specific areas, mostly along meadows, draws, and lake shores. We conclude that outbreaks of the disease require certain weather conditions or specific microclimatic situations in order for heavy infection to occur. Unfortunately, we do not know what the combination of factors are that result in periodic outbreaks. Additional research is needed to clarify the relationship between weather conditions and disease outbreak. Knowledge of past outbreaks does allow the forest manager to delineate sites of "high disease hazard", however.

During the study, we observed that lodgepole pine--a major host for the disease in British Columbia--was almost free of disease. From a strip sample through a mixed species stand, we noted only 5 lightly infected lodgepole pines out of 71 examined; whereas among 40 Jeffrey pines sampled, all were either moderately or heavily infected. It appears, therefore, that lodgepole pine may be highly resistant to Elytroderma disease in some areas.

## MANAGEMENT CONSIDERATIONS

Information about Elytroderma disease that should be of use in the management of Jeffrey pine stands include these major points:

1. Outbreaks of Elytroderma disease seem to occur most often on certain sites or in certain areas. These areas often can be delineated by past records of outbreaks. In addition, surveys and biological evaluations of present outbreaks will show the manager where "high risk" sites are located. Defining these high risk sites will at least give the manager the needed information on where the problem occurred before, where it is likely to occur again, and how serious the problem may be.
2. Effects of a single outbreak of disease can last for several years possibly resulting in heavy mortality and even substantial growth loss. Therefore, even though no further outbreaks occur for several years, the impact from the disease may be of major consequence in the management of some stands.
3. Weakening of trees by disease attack results not only in damage to trees from disease alone but also in damage and mortality from associated agents such as bark beetles.
4. Trees of all sizes, ages, and vigor classes are susceptible to attack and damage by Elytroderma disease. We know of no method of protection from or control of the disease in susceptible species. Planting or favoring existing non-susceptible species on high-risk sites is the best approach to control.

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## PENETRATION AND LATENCY IN NEEDLE CAST FUNGI

John M. Staley

In 1968, I presented some preliminary results of grafting studies using scions, the foliage of which had been exposed to spore showers of a Davisomycella sp. These scions were stripped of all but the 1965 foliage and grafted in March of 1966 to nursery run ponderosa pine seedlings. All scions came from a single mother tree that was consistently and very heavily infected by Davisomycella. On the mother tree, fruiting of the fungus was restricted to two-year-old and older foliage, and occurred in July of each year of observation from 1963 to 1969.

The grafted scions were subsequently held in a lath house or at other suitable locations in Fort Collins, far removed from any source of inoculum. The first fruiting of needlecast on the grafted scions occurred in 1967 and was restricted to the 1965 foliage. On the grafts, as on the scion mother tree in 1967, no symptomatic development was evident on either 1966 or 1967 foliage. In 1967 (and in subsequent years) all symptomatic foliage was stripped from most of the grafted scions prior to ascocarp maturity. On a few scions, ascocarps were allowed to mature on the symptomatic foliage, but such scions were removed to a safe distance from the lathhouse. It made no apparent difference in the results whether ascocarps were allowed to reach maturity on the grafted scions or not. In no year were symptoms apparent on any but the 1965 foliage of the grafted scions. Fruiting was observed on portions of this 1965 scion foliage in 1967, 1968, and 1969.

No fruiting was observed in the years subsequent to 1969, but two factors may be responsible. First, most or all of the 1965 foliage had been shed by 1969, and second, the years 1970 and 1971 did not provide very satisfactory weather for the development of ascocarps. The spring weather in those years was probably too warm and too dry. By 1971, all of the 1965 foliage was gone.

In 1967, symptoms developed on the scions held in Fort Collins, about 3 weeks prior to symptom development on the scion mother tree, which was about 3,000 feet higher in elevation than the lathhouse. There were no flecks or other incipient signs of disease present during the long latent period in the case of either of the scions, or the scion mother tree. The latent period was truly latent and not merely characterized by an obscure symptom development. It has thus been shown experimentally that the latent period for this needle cast may extend from two seasons to at least four seasons. Perhaps given different experimental circumstances, a longer latent period might have been observed.

Histological studies offered no suggestion of any systemic infection. In the case of this Davisomycella, hyphal growth is limited to a short band of needle tissue that is symptomatic. No invasion of phloem tissue such as that seen in the case of Elytroderma deformans can be detected, and no infection of foliage more recent than 1965 was observed as might have been expected with systemic infection of the grafted scion.

This grafting experiment also shows that the young developing foliage can serve as highly suitable infection court, even though it is separated from the sporulating foliage by an intervening foliated internode. My grafting experiment does not show whether or not the foliage on this intervening internode is additionally susceptible to infection, but artificial inoculations with a second species, D. ponderosae, indicate, in that case at least, a mild capacity of older foliage to serve as an infection court as well.

The precise location of the infection court is not clear for many species of the Hypodermataceae. We are left to assume that many of them, such as Virgella and Lirula, may operate essentially as do the Davisomycellas described above. However, in the case of several Lophodermias and one Lophodermium, which under natural conditions have a largely one-year disease cycle, it is all too clear that the primary infection court must be the newly developing foliage.

What happens at this infection court is a matter of concern for the full explanation of why needle casts operate as they do. Fortunately, we have a fairly complete idea of what happens in the case of one needle cast fungus, Lophodermium seditiosum.

The manner of penetration of this fungus has been demonstrated (1). The ascospores of Lophodermium seditiosum are produced in great abundance in Christmas tree plantations of infected Pinus sylvestris L. They are deposited in concentrations that exceed 2200 spores/cm<sup>2</sup>. Deposition is most dense on the distal third of the needle not protected by the subtending foliage, but ascospores are frequently detected at any random position on the needle. Symptoms develop first on the distal part of the needle. L. seditiosum casts the bulk of its ascospores at a time of year and under circumstances in which other Lophodermium species are largely inactive. It is on the overwhelmingly large ascospore production by L. seditiosum during the period of observation that my identification of the spores and appressorial penetration illustrated here depend.

Studies of ascospores deposited in water droplets on glass slides show the spores will sink to the bottom of such droplets. They do not, however, adhere to the glass surface as long as free water remains. Within one second following evaporation of the droplet, the spores fasten themselves so securely to the glass that they

cannot be moved without rupturing. It seems likely from this that spores are free to move about on wet pine needles and lodge in indentations between epidermal cells. Seldom, however, do they come to lie across a stomatal opening, and penetration even near stomates, so far as has been observed, is directly through the cuticle. Germination by germ tube alone has not been observed on leaf surfaces or on exposed spore trap slides. Germ tube germination can be seen in culture, but in nature, appressoria invariably develop.

At first, appressoria are hyaline and thin-walled. Thickening and melanization of the appressorial wall takes place in two or three weeks and judging from the initial lesion formation, penetration must occur at least by four or five weeks. Since fungicides largely prevent both appressorial melanization and symptom development, it appears that appressorial melanization is an essential step in the process of leaf penetration by L. seditiosum.

Sections cut from the distal one-third of needles revealed frequent penetration from melanized appressoria. Penetration hyphae grew directly through the cuticle and epidermis. In no case was penetration through a stomatal opening observed. Direct penetration through the cuticle near a stomatal opening did lead in at least one instance to hyphal growth into the substomatal chamber.

With these features of infection court activity having been demonstrated for Lophodermium seditiosum, it seemed logical to extend my observations to several needlecasts native to our western forests. This past season I therefore studied, at weekly intervals from July through October, the spore deposition and appressorial development of several Lophodermella species attacking lodgepole and limber pines. These were L. montivaga, L. concolor, and L. arcuata. In the process, I was able to make some observations on Bifusella sacatta. I expected these fungi to follow the familiar developmental pattern of Lophodermium seditiosum. Such was the case only to a certain point. The density of ascospore deposition was far more erratic than for L. seditiosum. It seemed to be more dependent upon a juxtaposition between the new foliage and the fruiting bodies borne on last years foliage than it was on other needle surface exposure factors. The position of developing lesions in the case of Lophodermella montivaga bore this out. The lesions which were evident in early September were frequent in the middle and lower thirds of the new needles and were not formed on the distally-exposed portions of the needles.

Upon germination, every spore observed (with the exception of Bifusella sacatta) produced an appressorium. These appressoria were only infrequently observed to thicken their walls, and in no case was appressorial melanization seen. The typical mode of germination can be seen in these slides.

No visual evidence relating to penetration of the needle was gained from this study. Occasional structures were observed in transverse section preparations which appeared to be appressoria, but no instances of penetration were seen. Some of these structures probably were appressoria as judged from attached, characteristically-shaped germ tubes. Others were probably spores of other leaf fungi.

The reason for lack of appressorial wall thickening and melanization remain unknown. One thing is clear, L. montivaga was nevertheless successful in penetrating the needle and causing lesions, even though this penetration was not observed histologically. Spores and appressoria could be visualized directly on the leaf surface by use of cotton blue in lactic acid. Although transverse sections were made through such visualized spore concentrations, penetration still was not observed.

In spite of this failure to obtain direct visual evidence, it seems clear that all the Lophodermellas studied, penetrate the leaf surface directly from appressoria. All Lophodermella spores observed germinated by appressorial formation, and it is inconceivable to me that some other rarely occurring process would lead to the successful disease production seen.

The case of B. saccata is more obscure. Its germ tubes were considerably thicker than those of any of the Lophodermellas. No distinct appressorium was formed although it is possible that the germ tube itself was a type of appressorium. It is also possible that B. saccata germinating on the youngest foliage was not developing in a suitable infection court. B. saccata attacks only the older needles on Pinus flexilis and perhaps it infects only through older needles. However that be, no attempt was made to observe spore germination on such older needles.

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ANNOSUS ROOT ROT IN SOUTHERN PINE  
BEETLE-INFESTED LOBLOLLY PINE STANDS

S.A. Alexander, J.M. Skelly, and R.S. Webb<sup>1/</sup>

The association of Heterobasidion annosus (Fr.) Bref. incidence and severity with southern pine beetle (Dendroctonus frontalis Linn.) infestations was examined among 476 loblolly pines (Pinus taeda L.) in Georgia, North Carolina, Texas, and Virginia. Twenty-two SPB infested and 18 noninfested BA-10 plots were established in loblolly stands in coastal plain, piedmont, and mountain regions on moderate to high hazard sites. Total height, height to live crown, c.b.g., crown rating, crown class, and shigometer readings were recorded for each tree prior to bulldozer excavation of the entire root system. Tree crowns were rated as dead (brown), thin and yellow, thin and green, or healthy. The presence of H. annosus basidiocarps for each tree and stump was recorded within each plot.

Once excavated, the primary and secondary roots of all trees with >1% colonization were measured as to the number, base diameter, and length of healthy and colonized roots. The length of healthy, resin-soaked, and stringy decayed tissue of each measured root was recorded with the latter two symptoms characteristic of H. annosus decay. A disc was removed at d.b.h. from each tree and the growth rate for the previous 0-to-10 years was determined. Shigometer readings were taken at d.b.h. from four points on the stem. Similar data was recorded for 5 paired tree plots in which two trees, one SPB-infested and one nearby which was uninfected, were excavated and analyzed.

Fifty-two SPB-infested and 29 noninfested trees were measured for which the average colonization incidence was 21 and 13 percent, respectively. SPB-infested and noninfested trees exhibited 47 and 14 percent colonization by length, respectively which was significant at  $P = 0.001$ . Secondary roots were colonized 44 and 13 percent for SPB-infested and noninfested trees, respectively, which was significant at  $P = 0.01$ . The number of colonized roots for SPB-infested and noninfested trees was 68 and 32 percent, respectively, which was significant at  $P = 0.001$ . A significant difference between infested and noninfested trees within the SPB plots indicated that H. annosus is positively associated with the southern pine beetle within the immediate infestation area. Radial growth reduction of both the previous 0-to-5 and 6-to-10 years increments was significantly and negatively correlated with both the percent number of roots and percent length colonized by H. annosus at  $P = 0.05$ . Significant  $R^2$  values were observed when shigometer readings were regressed against both 0-to-5 and

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6-to-10 years growth. Within the 5 paired tree plots, the average percent colonization was 46 and 15 for the SPB-infested and noninfested trees, respectively. Clearly a significant relationship has been established between H. annosus colonization and SPB infestation on moderate to high hazard sites in the southeastern U.S..

## A NEW MALADY OF WESTERN ASPEN

Thomas E. Hinds

Over the past several years we have been noticing individual and small groups of aspen in Colorado in which the branches droop similar to weeping willows. Upon finding tree mortality associated with this droopy syndrome, our observations and interest has become more intense for we find these abnormal trees scattered throughout the state; particularly along roadsides, in campgrounds, and as aspen transplants in urban areas.

The term "drooping aspen" is fairly descriptive of the disorder. Flexuous-rubbery pendant branches in the lower crown of large trees and throughout the entire crown of smaller trees with a lack of lateral twig growth for the past 5 to 25 years, and larger than usual sized terminal leaves but no lateral foliage on the branch behind the terminal foliage, characterize the malady. Buds appear to form normally on the current year's growth, but later turn black and fall off with the exception of the terminal bud. After 20 or more years, the pendulous branches die and eventually the entire tree succumbs. The symptoms do not appear to be clonal in nature nor entirely associated with man's activities.

Because of the pliant nature of the branches and lateral bud mortality, our first suspicion was that a virus might be involved. A cooperative agreement between the Rocky Mountain Station and Dr. Clark H. Livingston, Colorado State University, was made in 1976 for a preliminary study of the disorder. As results of the short term study were inconclusive and suggested that perhaps it might be a physiogenic disorder rather than an infectious disease, a new cooperative agreement was made for two years so that a more complete study, utilizing the services of a graduate student, could be accomplished. Results of the preliminary study will be forthcoming in the Plant Disease Reporter.

POTENTIAL OF NATURALLY INFECTED ROOT MATERIAL  
AS AN INOCULUM SOURCE FOR Phellinus weirii

J. D. Kellas<sup>1</sup>

INTRODUCTION

The use of naturally infected material as an inoculum source for Phellinus weirii (Murr.) Gilbertson has been virtually ignored. Early attempts of inoculating living trees using wood chips and agar permeated with P. weirii failed to infect the host roots (Buckland et al, 1954). Buckland and co-workers also reported using heavily permeated wood blocks as an inoculum source but only 2 of 36 inoculations infected previously damaged roots of Douglas fir (Pseudotsuga menziesii (Mirb.) Franco).

Attempts to infect Scots pine roots (Pinus sylvestris Linn.) with Fomes annosus (Fr.) Cke. using naturally infected root material resulted in low levels of infection of the host roots (Wallis, 1961). However, a successful technique was developed for inoculation using laboratory cultured F. annosus on sterile branch sections. This technique was subsequently adapted for inoculating Douglas fir with P. weirii (Wallis and Reynolds, 1962).

One technique has been reported for the mass inoculation of Douglas fir seedlings with P. weirii using naturally infected Douglas fir stem wood (Wallis and Reynolds, 1975). Seedling mortality was reported as early as five months following the introduction of the inoculum to the roots of one and two year old seedlings.

This paper reports a possible method for inoculating Douglas fir and western hemlock (Tsuga heterophylla (Raf.) Sarg.) roots with naturally infected root material and is part of a larger study investigating the growth of P. weirii on Douglas fir and western hemlock on several site-species combinations in the UBC Research Forest, Haney.

METHODS AND MATERIALS

Trial 1., February to May 1978.

Five Douglas fir and three western hemlock trees were inoculated with sections of Douglas fir roots naturally infected with P. weirii in February 1978. A total of 30 inoculations (22 and 8 respectively) were established. Infected root sections, bark intact and 1-5 cm diameter, were cut into 7.5 cm lengths and tied to previously excavated unwounded

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roots of the selected trees. The inoculations were covered with plastic sheeting and recovered with soil and organic matter.

Inoculations were inspected 9 weeks after inoculation to ascertain the number of inoculations where P. weirii had become established on the root of the host tree.

Trial 2., May to July 1978.

Three areas classified by Klinka (1976) as xeric, mesic, and hygric were selected containing 7 different site-species combinations of Douglas fir and/or western hemlock. A total of 210 inoculations were made on 105 trees; 60 Douglas fir and 45 western hemlock. Two sources of inoculum were collected, from Haney and from Surrey, and each tree was inoculated with each source of inoculum. The inoculum blocks were root sections 7-8 cm in length split longitudinally. Blocks showed advanced colonization of P. weirii with decay pockets well distributed through the root wood.

Roots of host trees were excavated and the inoculum blocks were attached with polypropylene rope and covered with plastic sheeting before the soil and/or organic matter was replaced. Roots inoculated were 1-8 cm in diameter. The inoculations of 35 trees were removed at 10 weeks and inspected for colonization of the host roots, brown crustose mycelial mat on the inoculum block and zone line formation.

Concurrently the two sources of inoculum were raised on 2% and 5% agar to study their rates of lineal growth under conditions of continuous light or darkness. Possible racial differences between the two sources were tested by "cross-plating" mycelia from both sources on agar plates, treated under the same conditions as above.

Trial 3., August to October 1978.

Ten inoculations each on Douglas fir and western hemlock were established using only inoculum from the Haney source. The inoculum blocks were similarly prepared as in the second trial. Inoculations were inspected 7 weeks after establishment.

## RESULTS

Trial 1.

Mycelium developed on 18 of the 22 Douglas fir roots and on 6 of the 8 western hemlock roots from the respective inoculum blocks after 9 weeks. Development on the host roots varied from one or a few pockets of mycelium to those where the block was firmly attached to the host root by mycelium.

Necrotic bark was observed under the mycelium of 7 Douglas fir roots and 4 western hemlock roots. Culturing on 2% malt agar indicated the presence of *P. weirii* in the necrotic areas of several roots. One inoculation of a western hemlock root showed a large necrotic zone surrounded by what appeared to be a "reaction zone". *P. weirii* was isolated from the necrotic region, but the "reaction zone" proved sterile.

#### Trial 2.

Assessment at 10 weeks showed that 25 of the 70 inoculations had developed mycelium on the host root and that 64 of the 70 blocks had developed zone lines on or under the exposed wood surfaces. A large proportion of the blocks from the Haney source developed a brown crustose mycelial mat on these surfaces, whilst few of the blocks from the Surrey source exhibited such growth. Viable *P. weirii* was detected in 44 of 59 blocks when isolations were made from the interior of these blocks and cultured on 2% malt agar.

Table 1 compares the formation of zone lines, block viability and the presence of *P. weirii* mycelium on the host roots.

The growth characteristics of the two sources of inoculum were tested by observing their development on 2% and 5% agar. The results (Table 2.) show that the rate of lineal growth of both sources raised in the dark was greater than that grown in continuous light.

Under conditions of continuous dark, the growth on 5% agar for both sources was greater than growth on 2% agar, there being no significant differences between the sources raised on the same medium. The colour of the dark grown cultures was white or cream, with copious fine aerial hyphae which filled the 100 mm diameter plates after 15 days.

Light grown cultures were not only slower growing but were different in appearance. The amount and extent of aerial hyphae was much less than the equivalent dark grown cultures. The cultures from the Surrey source were a mottled brownish yellow and those from the Haney source were mottled a strong brown. After 18 days those cultures from both sources raised on 2% agar had significantly greater lineal growth than those raised on 5% agar. The rate of growth of the Haney source was significantly greater than the rate of the Surrey source on both 2% and 5% agar raised in the light.

Cross plating of the two sources of inoculum produced a reaction at the interface of the advancing zones. The boundary between the two sources raised in the dark on 5% agar was distinct with no merging of hyphae from the two sources. Hyphae of both sources were white to cream coloured. However, the interface between the two sources raised in the dark on 2% agar differed. Hyphae of the Haney source adjacent to the interface became a strong dark brown colour which often extended over

the entire surface, whilst the hyphae of the Surrey source remained white to cream coloured. The reverse side of the 2% agar plates showed a strong black "zone-line" at the interface between the two sources. Microscopic examination showed the presence of masses of setal hyphae in the brown coloured areas of the plates.

### Trial 3.

Inoculations were removed 7 weeks after establishment. All 20 inoculations had developed mycelium on the host roots. The amount varied from one or two small pockets of mycelia 2 mm in diameter to instances where the inoculum block was firmly attached to the host root by mycelium along the entire length and width of the block. Necrotic bark was observed in 8 instances and penetration to P. weirii to the cambium was observed in 4 of these instances.

### DISCUSSION

The results of the first trial indicated that with refinement, healthy roots could be inoculated with P. weirii using naturally infected material. Of 30 inoculations, 24 showed mycelial development from the inoculum block onto the host root. Penetration of host bark was observed in 11 instances. Both Douglas fir and western hemlock were colonized by P. weirii in similar proportions. It was observed that some inoculation sections where the infected wood had been placed in contact with the host root, that a more luxuriant mycelial growth occurred. In the two subsequent trials the inoculum blocks were root sections 7-8 cm long and split longitudinally with an exposed surface placed in contact with the host root.

These modified inoculum blocks proved very successful in the third trial when all 20 inoculations colonized the surface of the host roots of both Douglas fir and western hemlock. Moist soil conditions prevailed in both the first and third trials, however, the period during the second trial only 3.43" of rain fell. The period May to July was the third driest for that period in the 19 years that records have been kept at Haney.

Inoculum blocks inspected after 10 weeks in the second trial showed abundant zone line formation, with some loss of viability, particularly with blocks from the Haney source. Zone line development by P. weirii has been reported to be influenced by several factors including soil moisture conditions (Nelson, 1973). Viable P. weirii was only present in blocks showing zone lines as previously reported for wood blocks buried for any length of time (Nelson, 1973). However, inoculum blocks from the third trial were generally covered by a cream to brown mycelial layer and zone lines were only observed where coverage was incomplete.

Racial differences between the two sources of P. weirii in Trial 2 were evident when cross plated on 2% and 5% agar. A distinct boundary

developed with no intermixing of hyphae from either source with some colour differences and an obvious black zone line-like formation occurring in dark grown 2% agar cultures.

No explanation is attempted to explain why the lineal growth of cultures raised on 5% agar were greater than on 2% agar for dark grown cultures or why the reverse was observed for light grown cultures.

Differences between the two inoculum sources were noted at the end of the second trial. The Haney blocks showed prolific development of crustose mycelium on exposed wood surfaces, ectotrophic mycelium developed on more host roots and a lower proportion of the blocks contained viable P. weirii compared to the inoculum blocks from the Surrey source.

The annual rainfall near where the Surrey inoculum was collected is approximately 49" compared to approximately 88" at Haney. Precipitation and soil moisture conditions may have through time influenced differentially the tolerance of P. weirii to drought stress for the two sources.

For the short duration of the three trials it appears that Douglas fir and western hemlock are equally susceptible to infection by P. weirii, although more concern is expressed as to the potential damage to Douglas fir than for western hemlock.

Immediately adjacent to where the Haney source was collected, many juvenile western hemlock trees remain free from P. weirii attack, yet trees close by were suitable for ectotrophic mycelial growth from inoculum blocks in the third trial.

Several questions are posed by the results so far:

- once zone lines have formed in an inoculum block, can P. weirii subsequently infect a host when suitable conditions prevail.
- are the racial differences between the two sources of inoculum attributable to drought tolerance?
- if summer is unsuitable for inoculations yet spring and fall are, when is P. weirii growing most actively?
- if Douglas fir and western hemlock appear equally susceptible during artificial inoculation, why isn't P. weirii of more concern in natural stands containing western hemlock?

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REPORT TO WIFDWC ON THE 5TH IUFRO CONFERENCE ON  
PROBLEMS OF ROOT AND BUTT ROT IN CONIFERS

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SUMMARY

Sponsored by the International Union of Forestry Research Organizations Working Group S-2.06-1 (Problems of Root and Butt Rot in Conifers). Held in Kassel, Federal Republic of Germany, 7-12 August, 1978. The conference was organized by the Hessian Forest Research Station at Hann Münden with Dr. Dimitri in charge of arrangements. Meetings were held in the city hall of Kassel, the Bürgersaal des Rathauses. 80 participants were registered from 23 different countries.

Gordy Wallis was to present a synopsis of this meeting to WIFDWC as he is the current chairman of the IUFRO working group on Problems of Root and Butt Rot in Conifers. However, as Gordy is not here, I have been asked to present a short summary of the meeting.

CONFERENCE FORMAT

The four previous IUFRO conferences sponsored by this working group dealt specifically with Fomes annosus. At the Kassel meeting the structure was changed with approximately 40% of the time devoted to F. annosus, 40% to Armillaria, and 20% to other root and butt rot problems and general concepts of wounds and subsequent decay in trees.

Format for the presentations started with statement papers on the current status of F. annosus throughout Europe, the USA and Canada. These were followed by invited position papers covering: Pathogen Biology; Infection Phenomena; Effect of Edaphic and Environmental Factors; Resistance and Relative Susceptibility; Biological, Chemical, and Silvicultural Control. Additional short contributions covering individual disease situations or research projects completed the formal F. annosus program.

Presentations dealing with Armillaria followed. They had a similar format to those on F. annosus starting with statement papers on the status of the disease in Northern and Western Europe, Canada, Australia, and New Zealand. Papers concerning the disease in the USA and Africa were listed in the program, but not presented as the authors were absent from the meetings and copies of their text were unavailable. This was unfortunate as the material was of interest to all participants. Some information concerning Armillaria in the Pacific Northwest was presented in the control paper of Shaw and Roth.

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Invited position papers followed with the same titles as those for F. annosus. Additional short contributions, noting individual disease situations and current research projects, completed the Armillaria section.

The final session covered other root and butt rot fungi and wound-decay problems. Papers on Polyporus tomentosus in eastern Canada and Poria weirii in the Pacific Northwest were presented along with short statement papers on wound-decay in Europe and the USA. Several papers dealt with various aspects of wounding and decay phenomena including possibilities of genetic, biological, and chemical control. Alex Shigo followed with his presentation on the CODIT system (Compartmentalization of Decay in Trees).

Most, but not all, of the papers presented were printed in a booklet and distributed to the registered participants prior to the conference. To provide for presentation of material not submitted by the deadline, poster displays were established. These remained in place for the duration of the conference, allowing ample time to view the material and discuss it with the authors.

A business session concluded the formal meetings. It was decided that the next conference will be held in 1983 in Australia, probably at or near Melbourne. This location will allow participants to attend the 4th International Congress of Plant Pathology which is also scheduled for 1983 in Melbourne. Dr. Dimitri was selected as the new chairman-elect of the working group with Dr. G. Kile elected as the new vice-chairman.

The conference closed with a field trip where we saw some local forest disease problems associated with Armillaria sp., F. annosus, tree wounding and subsequent decay, and die-backs of unknown origin. The field trip ended with a festive dinner in a remote hunting lodge where all participants thoroughly enjoyed themselves by eating several skewers of barbecued wild pork and imbibing ample quantities of quality German brew. Throughout the conference the Germans were gracious hosts, arranging several social gatherings. These included viewings of the national art collection held in Kassel and a dinner hosted by the city of Kassel.

Following the conference, there was a 2-day excursion from Kassel to Munich. The trip was arranged for people who were also attending the 3rd International Congress of Plant Pathology, which was held in Munich the following week. This relaxing drive in a Mercedes touring bus allowed ample time for informal discussions and viewing the countryside.

#### CURRENT STATUS OF DISEASE PROBLEMS

Summarizing the current status of disease problems associated with F. annosus and Armillaria from the various geographic regions covered during the conference is difficult. Those wishing detailed information are referred to the official proceedings which should be available through IUFRO within the next year.

Of particular interest to me was the report from Scotland concerning the occurrence of F. annosus in Sitka spruce. Stump infection via airborne spores and subsequent fungal growth down through stump roots and onto roots of adjacent standing trees is occurring where this species is planted on former pine sites. While not yet quantified, infection levels may be as high as noted in the United Kingdom and elsewhere for other conifer species.

A general impression I gathered concerning the status of F. annosus in Europe was summarized in the paper presented by Dr. Delatour from France:

F. annosus is an important silvicultural factor in management of conifer stands and especially Norway spruce. Research in this field will not give spectacular results but should allow us to better tolerate F. annosus by minimizing losses. An important problem is the practical application of results; they should be translated into a management rule, specifying the action of the forester in different types of situations. These guidelines should take account of site, stand characteristics (density, age, growth rate...) and attack characteristics (intensity, growth in trees...).

Disease problems associated with Armillaria sp. appeared to be even more diverse than with F. annosus. There were several instances noted where the fungus was regarded as mainly a secondary problem on trees weakened by some other primary factor or event. However, there were also reports where the fungus was considered the primary agent of extensive damage.

One of the stops on the field trip examined a mixed planted stand of Serbian spruce and Douglas-fir. European larch had seeded in naturally, creating a dense, mixed species stand around 3 to 5 m in height. Armillaria was killing a high percentage (30%+) of the spruce, while little mortality was visible on either the larch or Douglas-fir. The fungus was performing a beneficial thinning as long as one was not concerned with the change in species composition. The forest manager was not so concerned as the spruce was considered the wrong tree for the site even without the losses to Armillaria. Thus while losses to Armillaria were highly visible they had little silvicultural relevance. The recommendation given by research personnel to the forester in charge: allow the public to enter the stand to cut spruce for Christmas trees.

#### GENERAL COMMENTS

Communication was somewhat of a problem as papers were written and presented in several languages. The written texts usually provided English summaries. The European participants, while possessing a far better command of English than the English speaking people of the European languages, still had difficulty in following rather rapidly spoken English. This situation somewhat hampered discussions. Informal interpreters provided English summaries when necessary and aided discussions as much as possible.

Generally, adequate time for discussion followed the presentation of each paper. Even with language difficulties these exchanges often provided further insight into the various topics. As with past conferences, questions and answers raised during discussions will be part of the official record of the conference and appear in the proceedings.

Some personal highlights of the conference: The relatively free exchange of ideas and information that flowed through the informal discussions with forest pathologists from around the world; experiencing the international brotherhood that was created during the dinner at hunting lodge; viewing German forests and management practices after reading so much about them.

SCANNING ELECTRON MICROSCOPY REVEALS DIFFERENCES IN SURFACE MORPHOLOGY  
BETWEEN BASIDIOSPORES AND CONIDIA OF FOMES ANNOSUS

Charles G. Shaw III<sup>1</sup>

E. R. Florance<sup>2</sup>

The assistance of Dr. D. Goheen, USDA Forest Service, State and Private Forestry, Region-6, Portland, Oregon, who supplied sporophores and cultures, is most appreciated.

ABSTRACT

Basidiospores and conidia of Fomes annosus were examined by light and scanning electron microscopy (SEM). Light microscopy did not allow for accurate and reliable separation between the two spore types. Contrarily, SEM clearly demonstrated that conidia have a relatively smooth surface whereas basidiospores are consistently ornamented with numerous echinulations. SEM also demonstrated a definite dissimilarity in the shape and location of the apicular structure on basidiospores and conidia. With these differences now apparent attempts can be made to examine airborne inoculum of F. annosus for the presence, absence and/or relative abundance of the two spore types.

Additional Key Words: Ultrastructure; spore ornamentation.

INTRODUCTION

When discussing airborne inoculum of Fomes annosus (Fr.) Karst., an important source of infection for root wounds, stem wounds, and freshly cut stumps of conifers, the term "airborne spores" is commonly used. This label has been necessary because the actual spore type has not been identified. Basidiospores have commonly been considered the predominate spore type in airborne inoculum with conidia considered unlikely to serve an important role in airborne dispersal (Rishbeth 1951, 1957). Conidia, however, are known to occur naturally on forest debris and freshly cut stump surfaces (Morris and Knox 1962; Kallio 1971; Hunt et al. 1976) and may colonize stump surfaces after inoculation, albeit less thoroughly than after inoculation with basidiospores (Kuhlman and Hendrix 1964). Thus, both spore types could potentially contribute to airborne inoculum.

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Published mycological descriptions of the two spore types present a mixed record with text and drawings in Gaumann (1928) suggesting that some conidiophore heads were basidial-like and may represent the ancestral form of basidia. Bondartsev (1953), Lowe (1957), Domanski et al. (1967), and Gilbertson (1974) all indicate that the surface of the basidiospore wall is smooth. Bondartsev also notes that basidiospores are distinctly flattened on one side. Pegler and Waterson (1968) indicate that the surface is slightly asperulate, an impression also gained from some of the drawings in Hartig (1874). Lowe states that basidiospores are apiculus in drawings, but do not note it in their written description. All descriptions generally agree that basidiospores are somewhat ovoid and measure approximately  $3.5-6 \mu \times 3.5-4.5 \mu$ .

Conidia occur on oedocephaloid heads and are described from cultures as subglobose to ovoid, apiculate,  $4.5-7.5 \mu \times 3.0-6.0 \mu$ . (Nobles 1948, 1965). This description is in general agreement with those of Campbell (1938) and Bakshi (1950, 1952).

Such descriptions have led pathologists to assume that the two spores were almost identical morphologically (Kuhlman 1969). Certainly descriptions of spores currently available do not allow for accurate and reliable separation with the light microscope between collections of detached spores. The objective of this study was to determine if the two spore types could be distinguished through characters observable with a Scanning Electron Microscope (SEM).

#### MATERIALS AND METHODS

##### Basidiospores:

Sporocarps of F. annosus were collected from forest sites in the Coast Range of western Oregon and on Kosciusko Island in southeast Alaska. The Alaskan specimens from which basidiospores were obtained are deposited in the mycological herbarium at Washington State University (WSP #62361 and #62362). Basidiospores were obtained by suspending sporocarps over SEM stubs in a humidity chamber at room temperature for 24 to 48 hrs. After humidity treatment, pieces of tissue less than  $1 \text{ cm}^2$  were also removed from the pore layer of the same basidiocarps for light microscopy and processing for scanning electron microscopy. Tissues to be processed for SEM were transferred to Flo-thru Tissue Carrier Vials (American Optical Co.) and dehydrated.

##### Conidia:

Two cultures of the imperfect stage of Fomes annosus were used. One originated from typically decayed wood in western Oregon and the other from airborne inoculum in southeast Alaska. Stock cultures were subcultured onto PDA and incubated at  $20^{\circ}\text{C}$  for one week. Three mm agar plugs containing sporulating mycelium were removed aseptically and placed in Flo-thru Tissue Carrier Vials for subsequent dehydration.

## Dehydration and Microscopy:

Dehydration of both spore types was carried out in an ascending series of 4 ethanol/distilled water solutions (25, 50, 75, and 100% ethanol) followed by an ascending series of 4 ethanol/trifluoroethane solutions (25, 50, 75, and 100% trifluoroethane). Spores and tissue remained in each solution fifteen minutes. After treatment in 100% trifluoroethane they were critical point dried (Cohen et al. 1968). At this point some spores and tissue were used for light microscopy while others were mounted on SEM stubs, shadowed with gold/palladium alloy, and subsequently viewed with either an AMR 1200 or AMR 1000 SEM. Photographs were taken on Polaroid PN 55 black and white film.

## RESULTS

In general, light microscopy revealed smooth surfaces for both spore types; even when viewed with a 1000x oil immersion objective. However, by using depth of field and fine focusing the impression was obtained that small echinulations were present on some basidiospores.

SEM clearly demonstrated that conidia have a relatively smooth surface whereas basidiospores are consistently ornamented with numerous echinulations. Echinulations on basidiospores range in length from 0.045  $\mu$  to 0.20  $\mu$ . Their size likely accounts for the general failure to observe them with the light microscope. The maximum resolving power of a light microscope under optimum conditions is approximately 0.20  $\mu$ .

SEM also demonstrates a definite dissimilarity in the shape and location of the apicular structure. The basidiospore apiculus is a more distinct projection from the spore surface, eccentrically located and measuring 0.36  $\mu$  in length. The apiculi on conidia are generally centrally located, and there is a smoother continuum with the outer periphery of the spore wall. The apiculi measure approximately 0.54  $\mu$ .

Measurements of the two spore types yield a mean size for conidia of 3.46  $\mu$  x 4.79  $\mu$  (21 observations) and for basidiospores 2.52  $\mu$  x 3.51  $\mu$  (28 observations). Conidia from the Alaskan culture were significantly longer ( $P < 0.01$ ) and wider ( $0.01 < P < 0.05$ ) than those from the Oregon culture. The number of spores obtained from basidiocarps collected in Oregon was too small to statistically compare the sizes of the basidiospores between the two collection sites. However, based on 28 basidiospore measurements and 21 conidia from both cultures there is a highly significant difference ( $P < 0.01$ ) in both length and width between the basidiospores and conidia. The sizes for both spore types observed here are generally smaller than measurements previously reported (see introduction). This difference is likely attributable to our SEM measurements being made on spores in a dehydrated condition. The previously reported measurements were made by light microscopy and the spores were undoubtedly hydrated.

## DISCUSSION

Approximately 15,590 species of Basidiomycetes have been classified. Some 200 of these species (1.3%) have been investigated ultrastructurally (Hess and Weber 1976). Approximately 33% of the ultrastructural studies on Basidiomycetes have been with Uredinales, 24% with Ustilaginales, and 43% with the Hymenomycetes and Gastromycetes (Hess and Weber 1976). The studies pertaining to Hymenomycetes have concentrated mainly on the Agaricales and to date few other studies have been published that deal with members of the Polyporales (Pegler and Young 1973).

The surface morphology and size difference between basidiospores and conidia of *F. annosus* are now readily apparent. Attempts now can be made to collect airborne inoculum and examine it for the presence, absence, and/or relative abundance of the two spore types. The medium of Kuhlman and Hendrix (1962) may provide a means for selectively collecting and germinating spores of *F. annosus*. Freshly germinated spores could perhaps be fixed for SEM examination by the techniques we used for conidia. Such methods may provide a means for investigating the role of the Oedocephalum stage of *F. annosus* in nature, an area of research where the need for concentrated study has been noted (Staumbaugh, et al. 1962; Bega, 1963).

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## FOREST DISEASE (AND INSECT) RECORDS COMPUTERIZED

John G. Laut

Reports and records of diseases, causal organisms, and insects, gathered through surveillance and detection surveys by the Colorado State Forest Service have been computerized to facilitate their use. FAMULUS, a program developed to handle literature compilations, was modified to meet our needs.

Information on each collection or report is organized by fields and can be searched, compiled, compared, or otherwise manipulated in a variety of ways. The fields, and information presently contained in each are:

1. "District": State, county, organization (e.g., CSFS, USFS), district name (e.g., Grand Junction (CSFS), or Pike N.F., San Carlos district), specific location (e.g., legal description or grid, etc. will be added to this field).
2. Date: date collected, laboratory sequence number.
3. Host: by scientific and/or common name.
4. "Name": the common name of the disease or insect (e.g. Dutch elm disease, dwarf mistletoe).
5. Species: scientific name of organism involved.
6. Description: basic stand information (e.g., urban forest, plantation, etc., average DBH, size class of trees, number of trees involved).
7. Symptoms: part of tree involved and descriptors of the problem.
8. Damage: more detailed descriptors.
9. Enclosures: what kind of sample or specimen was received with report.
10. History: stand information - previous disturbance by fire, logging, pests, weather, man, etc.

The system presently contains only CSFS records for 1978. Older records will be added as time is available. Our plans also include adding herbarium records from the Rocky Mountain Forest and Range Experiment Station.

Any organization that takes this type of data and requires periodic compilations is invited to participate or develop its own system. The basic program is extremely flexible and, since no coding of field information is required, it is easy to operate and maintain.

## BIBLIOGRAPHY OF DUTCH ELM DISEASE

John G. Laut

The bibliography of Dutch elm disease, published in 1976<sup>1</sup> with addendum in 1977, has been continuously updated since that time. It now has been computerized utilizing the FAMULUS system<sup>2</sup> for information retrieval. It presently contains over 2,400 citations, many of which are accompanied by abstracts. New citations are added weekly.

A detailed users guide is being prepared but in the interim potential users can obtain searches and print-outs for any DED-related subject. The information is organized into several fields to facilitate retrieval. Searches of one or more of the fields can be made for broad or specific subjects. Persons requesting information should decide as precisely as possible what information is desired.

Requests for searches can be made to John G. Laut, Colorado State Forest Service, Colorado State University, Fort Collins, Colorado 80523, telephone (303) 491-6303.

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<sup>1</sup> Laut, J.G. and M.E. Schomaker. 1976. Dutch elm disease - A bibliography. CO State Forest Service, Ft. Collins. 135 pp (with addendum 1977).

<sup>2</sup> USDA-Forest Service. 1969. FAMULUS: A personal documentation system - A users manual. USDA For. Serv. Pacific SW For. and Range Exp. Stn., Berkeley, CA. Misc. Publ. 33 pp.

## NEW AND MODIFIED PROJECTS

### USDA Forest Service

#### Region 1

1. Dwarf mistletoe loss assessment in lodgepole pine in Montana (O. Dooling).
2. Expanded survey for root disease centers on three National Forests on about four million acres (R. Williams).
3. Dynamics of root disease centers and environmental factors correlated with centers (R. Williams).

#### Region 2

1. Survey of the incidence and damage caused by conifer broom rusts in Colorado (D. Johnson and L. Gillman).
2. Dwarf mistletoe loss assessment survey - Bighorn and Shoshone National Forests, Wyoming (D. Johnson, F. Hawksworth, D. Drummond).
3. Evaluation of jack pine mortality on the Nebraska National Forest (R. James).
4. Fomes annosus on white fir in Colorado (R. James).
5. Evaluation of salt damage to conifers along roadsides in Colorado (R. James).
6. Evaluation of air pollution effects on ponderosa pine in the Colorado Front Range (R. James and J. Staley).
7. Fungicidal tolerance of Botrytis cinerea (L. Gillman and R. James).
8. Evaluation of insect and disease conditions at Mesa Verde National Park, Colorado (R. James and K. Lister).

#### Pacific Northwest Forest and Range Experiment Station

1. Expanded field plot study (into SW Oregon) of Douglas-fir dwarf mistletoe development in thinned precommercial stands (D. Knutson) (modification of 73-F-2, Impact of dwarf mistletoe on Douglas-fir)
2. Inhibition of Fomes annosus on western hemlock stem disks by a Streptomyces sp. (C. Li).

3. Survival of Phellinus weirii in residual roots following stump removal and nitrogen fertilization (W. Thies).
4. Occurrence of Phellinus (Poria) weirii beyond visible limits of infection (W. Thies).
5. Growth loss of Douglas-fir infected by Phellinus weirii (W. Thies).

Pacific Southwest Forest and Range Experiment Station

1. Control of dwarf mistletoe-caused losses in young true fir stands by thinning (R.S. Smith and R.F. Scharpf).
2. Reduction in stem volume of grand firs defoliated by western spruce budworm outbreaks on the Payette National Forest, Idaho (G. Ferrell and R.F. Scharpf).
3. Greenhouse and nursery pathogenicity and symptomatology of four soil-borne fungi on five commercial species of conifers at various ages of growth (R.V. Bega).
4. Chemical and biological control of sugar pine root diseases at U.S.F.S., Placerville Nursery using seven fungicides and one suppressive soil (R.V. Bega).

(continued but never reported)

5. Population dynamics of dwarf mistletoe on true firs in California (R.F. Scharpf and J.R. Parmeter, 15- and 20-year readings).
6. The effect of dwarf mistletoe on mortality and volume loss in released true fir stands (R.F. Scharpf).
7. Reduction of dwarf mistletoe-caused mortality of Jeffrey pines by broom pruning (R.S. Smith and R.F. Scharpf).
8. Effect of thinning on the incidence and impact of cytospora canker, fir engraver beetle, and Fomes annosus in white fir stands on the Eastside Sierra Nevada (E. Wood, G. Ferrell, R.F. Scharpf, and J.R. Parmeter).
9. Lab, greenhouse, and nursery tests on effect of six mycorrhizal fungi on five species of conifers (R.V. Bega).

## States

### Washington State Department of Natural Resources

1. Near Glenwood, Washington, a series of plots are being established to test chemical control of Armillaria. We have not yet decided whether to control the disease within the stump or within the soil. Several types of fumigants will be used (K.W. Russell).
2. We are reexamining a series of Fomes annosus plots on the north end of the Olympic Peninsula near Clallam Bay. The plots were precommercially thinned in 1967 and treated with borax, Tordon, and Silvistar. We are looking at old stumps and crop trees to see how far Fomes annosus penetrated the standing trees (D. Chavez, C. Driver, R. Edmonds, and K. Russell).

## Universities

### University of Idaho

1. Suzanne Dubreuil is beginning her dissertation project on root disease caused by Phaeolus schweinitzii.
2. A new project was initiated last year to study cavity-nesting birds in decayed dead or alive standing trees.
3. Collection and identification of wood-inhabiting fungi under a grant by the Stillinger Fund.
4. The field work is already finished on a study of mortality centers in the southern end of the Northern Rockies, Boise N.F.; conducted for the U.S. Forest Service.

## TERMINATED PROJECTS

### USDA Forest Service

#### Region 2

1. Mycorrhizal inoculation of container-grown Engelmann spruce and lodgepole pine (D. Hildebrand).

#### Region 6

1. Evaluation of tree species susceptibility to laminated root rot in eastern Oregon and Washington (G. Filip).
2. Evaluation of blue stain penetration and checking in recently killed lodgepole pine in northeastern Oregon (R. Harvey and J. Hadfield).
3. Evaluation of truben and barrot for disease control at the Westfir Nursery (D. Goheen).

#### Pacific Southwest Forest and Range Experiment Station

1. 63-F-2
2. 71-K-1 (change to C.S. Hodges and J. Kliejunas)
3. 72-K-1 (change to C.S. Hodges and J. Kliejunas)

### States

#### Washington State Department of Natural Resources

1. Completed a moss control test for container seedlings using X-77 spreader and captan fungicide. See Disease Control Committee report (K.W. Russell).
2. Completed an IR-4 fungicide evaluation test for container seedlings. Captan emerged as one of the best fungicides for control of Botrytis. Routine captan sprays as a preventive were almost 100% successful as a deterrent to Botrytis (K.W. Russell).

## Universities

### University of Idaho

1. David Kulhavy has finished his work on insect/disease associations in western white pine and lodgepole pine. He is now writing the results.
2. A student laboratory manual "Forest Pathology Outline" was finished and published last year.
3. Another publication, "keys to major disease, insect and related problems of forests in Northern Idaho" was published in 1977 but supplies were exhausted. A revised edition was published in the summer of this year.
4. Field work is finished on effects of artificial defoliation on grand fir. This project began in 1975 as announced at the Missoula meeting. Analyses are now in progress.
5. William Livingston finished his work (MS degree) on Pseudohylesinus granulatus as possible vectors of root diseases in grand fir.

WESTERN INTERNATIONAL FOREST DISEASE WORK CONFERENCE

DWARF MISTLETOE COMMITTEE REPORT

HIGHLIGHTS OF 1978 DWARF MISTLETOE RESEARCH

John G. Laut, Chairman

I. Taxonomy, Hosts, and Distribution

- a. Arceuthobium cyanocarpum, a common parasite of Pinus flexilis, was found for the first time on whitebark pine, Pinus albicaulis, in Wyoming. In an area near South Pass in the Wind River Range, the parasite was common and damaging on both limber pine and whitebark in a mixed stand (F. G. Hawksworth, RM Station, Fort Collins, and Dave Johnson, Region 2, Denver).
- b. A morphological study of the pollen grains of Arceuthobium, using light microscope and SEM techniques has been started. Analyses of all 38 known dwarf mistletoes (from both the Old World and New World) are being made to determine if individual species can be identified by their pollen morphology (R. Mathewes, Simon Fraser Univ., Burnaby, B.C., and F. G. Hawksworth, RM Station, Fort Collins).
- c. Branching type in all U. S. dwarf mistletoes (except A. americanum = subgenus Arceuthobium) has been thought to be exclusively fan-shaped (flabellate = subgenus Vaginatium). However, a study of 30 populations of A. occidentale and 33 of A. campylopodium in California showed that all but 1 had some whorled branching. About 4 percent of both species had shoots with whorled branches; the remaining 96 percent were flabellate. From a taxonomic standpoint, we still feel that branching types are valid for separating the two subgenera of Arceuthobium. However, the presence of flabellate branching, rather than the absence of whorled branching, is a better criterion for distinguishing subgenus Vaginatium. Subgenus Arceuthobium seems to have only whorled branching (W. R. Mark, Cal. Poly State Univ., San Luis Obispo, and F. G. Hawksworth, RM Station, Fort Collins).

- d. First collections of A. americanum on lodgepole pine were made from the Castle Mountains in Meagher County and the Pryor Mountains in Carbon County, Montana. The specimens were deposited in the Mistletoe Herbarium in Fort Collins (O. Dooling, Region 1, Missoula).
- e. Approximately 233 miles of spruce cover type in 28 counties in the lower peninsula of Michigan were surveyed for eastern dwarf mistletoe. The parasite is not prevalent or damaging in any of the counties surveyed, and was present in less than 1 percent of the spruce stands examined. In most instances mistletoe infestations consisted of only a few infected and/or dead trees. Infected black spruce were consistently located in spruce-cedar bogs and only one infestation in white spruce was detected (R. L. Mathiasen, Michigan State University).
- f. A manuscript has been accepted for publication in The Southwestern Naturalist, "Distribution and effect of dwarf mistletoes parasitizing Pinus strobiformis in Arizona and New Mexico," and should appear in February or March, 1979. Arceuthobium apachecum and A. blumeri parasitize Pinus strobiformis in the southwestern United States. The mortality rate of this host in mixed conifer stands heavily infested with A. apachecum was 30 times greater than in non-infested stands and 20 times greater in stands heavily infested with A. blumeri. Surveys in southern Colorado, northern New Mexico, and central Arizona did not detect additional populations of A. apachecum although P. strobiformis was present in the areas surveyed. Analysis of morphological characters of the dwarf mistletoe population parasitizing P. strobiformis in the Santa Rita Mountains, Arizona indicate the parasite is A. apachecum, and not A. blumeri as reported previously. A. blumeri is only known in the United States from the Huachuca Mountains, Arizona but extends south to Durango, Mexico, and is reported from Sonora, Mexico for the first time. Contrary to a previous report, A. blumeri frequently causes the formation of witches' brooms on P. strobiformis. Pinus ayacahuite var. brachyptera is recorded as a host for A. blumeri in Durango, Mexico for the first time (R. L. Mathiasen, University of Arizona).

- g. Arceuthobium gillii subsp. gillii, A. blumeri, and A. vaginatum subsp. cryptopodum have been collected from Sonora, Mexico in the Sierra de Ajos approximately 20 miles southeast of Cananea. This represents the first report of A. blumeri in Sonora, but only the second collections of the other two taxa from Sonora (R. L. Mathiasen, University of Arizona).
- h. During this year, we discovered Arceuthobium campylopodum growing on Norway spruce. The host is located in Chelan State Park on Chelan Lake, Washington (K. Russell, Wash. DNR, Olympia).
- i. Field tests were begun in the winter of 1977-78 to test the host parasite relationships of the Arceuthobium campylopodum -- occidentale complex. Cross inoculations with seeds of several collections of both A. campylopodum and A. occidentale from several hosts were made on several pine species including ponderosa pine, Jeffrey pine, knobcone pine, Coulter pine, digger pine, and Monterey pine. Tests were made in the native ranges of the host species which include both the Sierra Nevada and the Coast range of California (W. Mark, Cal. Poly State Univ., San Luis Obispo, and R. F. Scharpf, PSW, Berkeley).

## II. Physiology and Anatomy

- a. A normal phloem has long been regarded as being absent from the dwarf mistletoes, Arceuthobium. Now, our fluorescence microscopy studies reveal that a primitive member of the genus, A. globosum, has highly specialized phloem tissue. The sieve tube members of this large Mexican species have transverse to oblique sieve plates, and small but numerous diffuse lateral pores (C. Calvin, Portland State Univ., F. G. Hawksworth, RM Station, Fort Collins, and D. Knutson, PNW Station, Corvallis).
- b. Germination, respiration, and photosynthesis in seeds of dwarf mistletoe (Arceuthobium) were studied. The effects of 1 hour soakings of seeds in aqueous solutions of 1, 2, or 3 percent H<sub>2</sub>O<sub>2</sub> or 1, 2, 3, 4, or 5 percent Chlorox on germination of seeds were tested. Germination rates for seeds from three consecutive years (1975-1977) were obtained. Germination rates varied widely. This variance transcended subtle changes in treatment. Pre-treatment of seeds with Chlorox resulted in significantly

lower germination rates than those of the control. Chlorophyll concentrations were determined for seeds and aerial shoot tissue of four species. Seeds of A. douglasii had the highest chlorophyll concentration (0.38 mg/g fresh weight) while seeds of A. tsugense had the lowest concentration (0.25 mg/g fresh weight). Net O<sub>2</sub> uptake by seeds of A. campylopodum in dark and in light was measured by manometric and polarographic methods. The mean values were 353  $\mu\text{l O}_2 \text{ g}^{-1}\text{h}^{-1}$  in the dark and 201  $\mu\text{l O}_2 \text{ g}^{-1}\text{h}^{-1}$  in the light. The difference between these rates is apparently due to O<sub>2</sub> evolution during photosynthesis. In light the seeds can fix 43 percent of the CO<sub>2</sub> produced by respiration. Experiments in which seeds were exposed to <sup>14</sup>C<sub>2</sub>O in light confirmed that the seeds are able to fix CO<sub>2</sub>. Extraction of seeds with ethanol showed that 97-99 percent of the incorporated <sup>14</sup>C was ethanol soluble. Ten to 16 percent of the ethanol fraction was chloroform soluble while the rest was H<sub>2</sub>O soluble. Ion exchange separation of the H<sub>2</sub>O phase showed that 11-25 percent of <sup>14</sup>C activity was cationic, 15-19 percent anionic, and 53-67 percent neutral (S. W. Gustafson, Portland State Univ., Portland).

- c. Work is in progress on the anatomy of the aerial epidermis of A. tsugense and certain other dwarf mistletoes (Calvin, PSU, Portland).

### III. Life Cycle Studies

- a. Dwarf mistletoes (Arceuthobium spp.) have been thought to spread mainly by means of explosive fruits that expel seeds under hydrostatic pressure to distances up to 130 feet. Recently, birds and mammals have been considered possible agents in long distance dissemination of the seeds. This study investigates the role that small mammals, especially the red squirrel (Tamiasciurus hudsonicus), may play in seed dispersal. The study was conducted in the Malheur National Forest on Graham Creek near Prairie City, Oregon. Mistletoe species present were A. campylopodum, A. douglasii, A. laricis, and A. americanum, and primary host species of each were Pinus ponderosa, Pseudotsuga menziesii, Larix occidentalis and Pinus contorta respectively. Two separate areas, A and B, were studied and characterized for species composition and extent of mistletoe infection. A study area in Area B was established

for observing behavior and movement of red squirrels. Squirrels were trapped and shot in each area when the seeds began to erupt. The two stands varied slightly in species composition and drastically in their degree of infection. No seeds were found on trapped or shot squirrels in Area B where infection was low. Fifty percent of the squirrels trapped or shot in Area A carried seeds on their fur. It appears that squirrels do carry mistletoe seeds over distances up to 150 m. if their territory is in a stand that exceeds a threshold level of infection. The number of seeds carried per year can be predicted. It is doubtful whether a significant number of infections result from squirrel dispersal of seeds since most seeds carried on the fur are probably groomed off in an uninfected part of the host tree (D. E. Lemons, Portland State Univ., Portland).

#### IV. Host-Parasite Relations

No reports.

#### V. Effects on Hosts

- a. Effect of dwarf mistletoe on growth of released red firs in California. The manuscript is still in the editor's shop (R. F. Scharpf, PSW, Berkeley).
- b. The manuscript "Dwarf mistletoe does not increase trunk taper in released red firs in California" has been published. USDA, Forest Service Res. Note, PSW 326, 1977 (R. F. Scharpf, PSW, Berkeley).
- c. Examination of permanent plots in summer of 1978 showed that neither uninfected red firs nor firs severely infected with dwarf mistletoe suffered mortality during the severe drought in California in 1976 and 1977. Less than 1 percent of the plot trees had died since 1973, and none of the deaths could be directly attributed to either drought or dwarf mistletoe (R. F. Scharpf, PSW, Berkeley).
- d. With the discovery of additional 1974 infections during the 1978 spring check of planted western hemlock at Nitinat, the average upward advance of A. tsugense for 1974 is revised to 19.1 cm from 13.6 cm noted in the 1977 report. Average height growth of the trees in 1974 was 34.7 cm. Infections from 1975 seed resulted in an average upward spread

of 17.6 cm but this is likely to be increased as "new" 1975 infections are found in the spring of 1979. In the first three to four years following overstory removal in 1969, there was a marked decrease in percentage of crown infected. This percentage is now holding steady at 30-50 percent, the actual level depending on the original degree of infection (R. B. Smith, PFRC, Victoria).

## VI. Ecology

- a. A survey was conducted to assess the distribution and effects of the mistletoes on the pinyon-juniper vegetation type on the south rim at Grand Canyon National Park, Arizona. Two mistletoe-host relationships -- Arceuthobium divaricatum Engelm. on Pinus edulis Engelm. (spread by forceful ejection) and Phoradendron juniperinum Engelm. on Juniperus osteosperma (Torr.) Little (spread by birds) -- were investigated. Both mistletoes were present throughout the park. The only areas devoid of mistletoe infection were those areas with a history of a burn. Fire was the most limiting factor in the distribution of the mistletoes. The incidence of infection increased as trunk diameter and height increased in both pinyon and juniper. Arceuthobium divaricatum had the most serious impact on its host of the two mistletoes in respect to both vigor and mortality. Both mistletoes appear to be in equilibrium with their hosts at this time. A control program does not appear warranted, but perhaps for aesthetic purposes may be considered in the future (Alyce Hreha, Provo, Utah).
- b. The last measurements of field inoculations (see 1971-73, 1975 and 1976 reports) were made in the spring of 1978. A manuscript, "Infection Trials with Three Dwarf Mistletoe Species Within and Beyond their Natural Ranges in British Columbia" is undergoing local editorial review (R. B. Smith, PFRC, Victoria).
- c. Terry Shaw and I are conducting a cooperative study to determine how past old-growth timber harvesting has affected the occurrence and distribution of dwarf mistletoe in the developing young-growth stands. To determine if there is now a mistletoe problem in the young-growth stands studded with infected residuals. We have had field

crews out this summer and will have more out next summer. The study will be finished next fall (T. Shaw, Region 10, and T. Laurent, Forest Service Lab, Juneau).

#### VII. Control - Chemical

No reports.

#### VIII. Control - Biological

- a. L. A. Kelton and J. C. Herring (Can. Ent. 110: 779-780, 1978) describe two new species of plant bugs, Neoborella (Miridae) collected on Arceuthobium. This brings the total to four species known to live on dwarf mistletoe. A key to separate the North American species of Neoborella is provided. The article describes N. canadensis new sp. on Arceuthobium americanum, on Pinus banksiana (Bellis, Alberta, Canada, also at Meadow Lake), and N. pseudotsugae new sp. on Arceuthobium douglasii on Pseudotsuga menziesii (Humphrey's Peak, Flagstaff, Arizona, also at Hannagan, Arizona, and Cloudcroft, New Mexico).
- b. Morphological and biological characteristics of a lodgepole pine dwarf mistletoe-associated rust, Peridermium betheli Hedgc. and Long, were evaluated and compared to the blister rust, Cronartium comandrae Peck, in an attempt to determine the taxonomic status of P. betheli. Aeciospores of P. betheli were found to be shorter and more globose than those of C. comandrae. Morphology of the aeciospore wall as observed under a scanning electron microscope was similar for both rusts. Aeciospore germination characteristics were similar for P. betheli and C. comandrae. No P. betheli hyphae were observed in histological examinations of lodgepole pine seedlings naturally infected with dwarf mistletoe and inoculated with P. betheli in the greenhouse. P. betheli sporulation period was found to be later in the season than C. comandrae. P. betheli can cause tree mortality; however, its damage was not as severe as that caused by C. comandrae. The distribution of P. betheli was mapped and found to extend through the Rocky Mountains and one location in the Sierra Nevadas. P. betheli distribution coincides with the range of A. americanum on lodgepole pine. The results suggest that P. betheli is a heteroecious ecotype of C. comandrae. Conclusions regarding the taxonomic status of P. betheli require additional research (C. Dixon and W. Nishijima, Colo. State Univ., and F. G. Hawksworth, RM Station, Fort Collins, and R. G. Krebill, RM Station, Tempe).

- c. One of the first attempts to breed trees for disease resistance was begun in the early 1920's in Colorado for mistletoe-resistant ponderosa pine (C. Bates, J. Forestry 25: 130-144, 1927). Two plantations of putatively resistant and susceptible strains were established near Colorado Springs in 1932. The plantations were relocated in 1978. Some "resistant" trees were found to be infected, but we were not able to make a detailed comparison of the amount of mistletoe on the "resistant" vs. "susceptible" strains. Further examinations are planned in the plantations next spring (F. G. Hawksworth, RM Station, Fort Collins).
- d. A. tsugense infections sprayed with Nectria macrospora (fuckeliana) in the Jordan River (Vancouver Island) area (see 1973 report) resulted in no definite takes. A new trial was established in the Cowichan Lake area also on Vancouver Island on October 22, 1975. Of 20 infections sprayed, 8 produced N. macrospora conidial or perithecial structures and associated symptoms of cankering and resinosis -- apparently a successful trial. However, of 20 unsprayed mistletoe infections, 2 also produced N. macrospora signs. All infections have been removed and are being examined in the lab (A. Funk and R. B. Smith, PFCR, Victoria).

#### IX. Control - Silvicultural

- a. To determine the effects of thinning in mistletoe-infested ponderosa pine and lodgepole pine stands, a series of permanent plots (mostly 0.4 acre) are being established. Stands with no mistletoe and those with different intensities of infection are being thinned to various growing stock levels. We plan to evaluate the mistletoe and tree responses to thinning. To date, about 80 plots have been established: 65 in ponderosa pine (in Colorado and Arizona) and 15 in lodgepole pine (F. G. Hawksworth, RM Station, Fort Collins).
- b. The proceedings of the symposium "Dwarf mistletoe control through forest management," held in Berkeley in April 1978, is available -- USDA, Forest Service, General Technical Rep., PSW 31, 1978. Although emphasis is on silvicultural control, aspects of

chemical, biological and genetic control are also discussed. The symposium was organized around the following general topics: bases for control, control planning and decision making, control operations and accomplishments, refining and improving control techniques, pest damage and integrated control, and needed research (R. F. Scharpf, PSW, J. R. Parmeter, U. C.).

- c. U. S. Forest Service, Region 5 and PSW began a pilot test project in the summer of 1978 to test the efficiency of control of dwarf mistletoe by thinning in pre-commercial stands of true firs. Infested stands in need of thinning are now being located and selected for plots in California's National Forests (R. F. Scharpf, PSW, R. S. Smith, Region 5).
- d. We have 32 plots that were established in 1974 in pre-commercially thinned units. They are designed to evaluate success of operational thinning programs in eastern Washington. They will not be remeasured until 1979. Our original measurement showed good control (K. Russell, Wash. DNR, Olympia).
- e. Removed A. americanum infected overstory lodgepole pine trees from 5,746 acres on 4 central Montana National Forests (O. Dooling, Region 1, Missoula).

#### X. Surveys

- a. Dwarf mistletoe loss assessment survey -- Medicine Bow National Forest in Wyoming. As part of an effort to assess growth loss and mortality caused by dwarf mistletoe, Arceuthobium americanum, in western coniferous forests, two approaches were used to estimate annual cubic foot volume loss in lodgepole pine forests on the Medicine Bow National Forest in Wyoming. The first approach was to use data derived from a 1970-71 Stage 1 Timber Inventory (available from Timber Management, Rocky Mountain Region), and the second one was to conduct a road-plot survey to estimate dwarf mistletoe incidence and associated volume loss. Results indicate that existing mistletoe data in timber inventory files are inadequate when trying to develop growth loss information. Suggestions for improvement of survey data gathering are presented. A similar survey was

conducted on the Bighorn and Shoshone National Forests during the 1978 field season. Results will be available in late 1978 (D. W. Johnson, F. G. Hawksworth and D. B. Drummond; Region 2, RM Station and MAG, USFS). Report available from USDA, Forest Service, Methods Application Group, Report No. 78-1, 6 pp.

- b. A survey of the incidence and infection level of lodgepole pine dwarf mistletoe was conducted on the Red Feather Ranger District, Roosevelt National Forest, during 1973, 1974, 1975 and 1977. Variable radius plots (BAF 10) were located on a 10 by 10 chain grid. Understory data were collected on fixed radius plots (0.01 acre). Over 7,300 plots were established on 45,000 acres. Dwarf mistletoe was recorded on 83.8 percent of the plots that fell within lodgepole pine type. Over 53 percent of the plots had an average DMR of 3 or greater. Data were input into the RMYLD Program to determine growth loss and mortality associated with dwarf mistletoe infection. It has been estimated that the annual volume loss is 685, 450 cubic feet for the Red Feather District. This is an average yearly loss of 15.2 cubic feet per acre. A final report of the survey will be prepared in late 1978 (D. W. Johnson, Region 2, USFS).
- c. Finished the field part of a survey to determine cubic foot volume loss due to A. americanum on lodgepole pine in central Montana. The survey covered the Beaverhead, Custer, Deerlodge, Gallatin, Helena, and Lewis and Clark National Forests. The data will be analyzed during the winter of 1978 (O. Dooling, Region 1, Missoula).
- d. A total of 3,040 acres of lodgepole pine forests in the Island Park area of eastern Idaho were surveyed from a Bell Helicopter cruising at 40-65 mph 100-200 feet above the forest canopy. Witches' brooms were readily discerned and could be used to distinguish general levels of infection (high, medium or low). The low cost (about \$.25/acre) and relatively high degree of accuracy indicate this would be a very cost-effective survey method in areas of widely fluctuating dwarf mistletoe infection levels. However, in this area of low stumpage values, high losses from the mountain pine beetle, and consistently medium to high levels of dwarf mistletoe infection, it is difficult to justify even this small investment (Report No. 78-6 Idaho Department of Lands, Coeur d'Alene, Idaho; J. Schwandt, Idaho Department of Lands).

## XI. Miscellaneous

- a. Masters thesis: A peridermium rust associated with dwarf mistletoe on lodgepole pine. Christine Dixon, Colo. State Univ., 100 p., 1978. (See abstract under VIII.)
- b. Masters thesis: Small mammal dissemination of dwarf mistletoe seeds. D. E. Lemon, Portland State Univ., 38 p., 1978. (See abstract under III.)
- c. Masters thesis: A comparative distribution of two mistletoes: Arceuthobium divaricatum and Phoradendron juniperinum (South Rim, Grand Canyon National Park, Arizona). A. Hreha, Brigham Young Univ., 62 p., 1978. (See abstract under VI.)
- d. Masters thesis: Germination, respiration, and photosynthesis in seeds of dwarf mistletoe (Arceuthobium). S. W. Gustafson, Portland State Univ., 48 p., 1978.
- e. The FAMULUS mistletoe information retrieval system is alive and well in Fort Collins and Berkeley. Since last year's W.I.F.D.W.I.C., we processed about 70 requests for literature, mostly from the western U.S. and Canada, but also from South Africa, Sweden, Switzerland, Germany, and Australia. A display of the system will be shown in Tucson (F. G. Hawksworth, RM Station, Fort Collins, and R. F. Scharpf, PSW Station, Berkeley).

## Root Disease Committee

Walt Thies, Acting Chairman

Discussion of the Root Disease Committee centered on the purpose and format of future meetings:

1. Greg Filip was elected chairman for the 1979 meeting.
2. Current root disease work will be summarized and mailed to WIFDWC members prior to the 1979 meeting. The summary will be produced from responses to a questionnaire to be mailed to the membership.
3. Discussion at the 1979 committee meeting will center on a narrow topic selected by the chairman and introduced by two speakers, each spending 5-10 minutes addressing the topic. These presentations will serve as a focus for the committee discussion.

Disease Control Committee

Highlights of 1978 Control Investigations

Kenelm Russell, Chairman

I. SEEDLING DISEASES

A. Damping off and animal control

Host: Ponderosa pine

Causal Organisms: Damping off fungi, birds and rodents

Control: Chemical

Development Stage: Field Trial

Petroleum mulch was used at two rates (100 & 200 gal/acre) to increase seed germination and protect seeds in seedbed from predation by birds and rodents. Results did not increase overall germination percent, but treated did germinate slightly faster. Birds were not observed in treated area. (R. Harvey, USFS - Region 6, Portland.)

B. Moss

Host: Container seedlings

Causal Organisms: Various Mosses

Control: Chemical

Development Stage: Pilot Operational

Developed successful treatment for moss in container cavities by using mixture of captan and X-77 surfactant. Very pleased with results. Complete story will be published in Winter 1979 in both Tree Planter's Notes and PDR. Material to have a 24(c) registration. (K. Russell, W. Haglund, WDNR & Cascade Agricultural Service Co., Olympia.)

C. Rosellinia

Host: Douglas-fir

Causal Organisms: Rosellinia herpotrichioides

Control: Chemical - Biological

Development Stage: Full Operational

An outbreak of the disease resulted in a test to see if the fungus would be a problem in storage. We sprayed several treatments with fungicides and stored trees for up to one year at 36° F, then outplanted. Findings were that fungicides not needed. Disease can be culled during packaging. No problem occurred when planted in pots. (K. Russell, WDNR, Olympia.)

D. Damping off & Root Disease

Host: Sugar pine

Causal Organisms: Fusarium, Pythium, Phytophthora spp.

Control: Chemical - Biological

Development Stage: Field Trial

Seven fungicides, one suppressive soil used. Started May, 1978 - Placerville, USFS Nursery. Results not yet available. (R. Bega, A. McCain, USFS & UC Berkeley.)

E. Damping-off

Host: Ponderosa pine  
Causal Organisms: Pythium, Fusarium  
Control: Chemical  
Development Stage: Field Trial

At Big Sioux Nursery, SD, fumigation with Dowfume MC-2 (400 lb/acre) gave good weed control. Damping off did not occur in either fumigated or non-fumigated beds. (Linnea Gillman, USDA Forest Service.)

F. Grey Mold

Host: Lodgepole pine  
Causal Organisms: Botrytis cinerea  
Control: Chemical  
Development Stage: In Vitro

Most Colorado Botrytis isolates were tolerant of benomyl in vitro. Dichloran completely inhibited fungus growth and captan and chlorothalonil slowed growth significantly. (Linnea Gillman & Bob James, USDA Forest Service.)

G. Damping-off

Host: Ponderosa pine, Engelmann spruce, Lodgepole pine  
Causal Organisms: Pythium, Fusarium  
Control: Chemical  
Development Stage: Operational

At Mt. Sopris, Nursery, Colorado, fumigation with Dowfume MC-33 (400 lb/acre) in September, 1977 resulted in a 4-fold increase in 1-0 ponderosa pine survival compared with pine grown in non-fumigated beds. (Linnea Gillman, USDA Forest Service.)

H. Botrytis Gray Mold

Host: Sitka spruce, Western hemlock, Douglas-fir  
Causal Organisms: Botrytis cinerea  
Control: Chemical  
Development Stage: Operational

We are comparing two fungicides applied through the irrigation system vs. normal spraying on a three week schedule. It looks like the irrigation application is not effective. (A. H. McCain, Univ. of Calif., Berkeley.)

I. Fusarium root rot

Host: Conifer seedlings  
Causal Organisms: Fusarium oxysporum  
Control: Chemical - Biological  
Development Stage: Pot Trial

Just begun. (David Adams, California Dept. of Forestry.)

- J. Grey Mould  
Host: Western hemlock  
Causal Organisms: Botrytis cinerea  
Control: Chemical  
Development Stage: Operational

A survey of proportions of Benlate sensitive and tolerant isolates showed all sensitive isolates at a new nursery; all tolerant at the oldest nursery; a mixture of types elsewhere. (John Hopkins, Pacific Forest Research Center, Victoria, BC.)

- K. Fusarium  
Host: Sugar pine  
Causal Organisms: Fusarium oxysporum f. sp. pini  
Control: Chemical  
Development Stage: Field Trial

Tested six chemicals for control of a Fusarium collar rot. It was not a good test of the chemicals because the disease levels were too low. (Michael Srago, Art McCain, USFS & UC Berkeley.)

## II. FOLIAGE DISEASES

- A. Swiss Needle cast  
Host: Douglas-fir  
Causal Organisms: Phaeocryptopus gaumanni  
Control: Chemical  
Development Stage: Field Trial

Field trials from 1977 were quite successful. Dithane M-45 and Benlate give good control. We are refining dosages and timing now. At least three applications are necessary. (B. Fatuga, K. Russell, WDNR Olympia and UW, Seattle, WA.)

## III. STEM DISEASES AND WILTS

- A. Dutch elm disease  
Host: Elms  
Causal Organisms: Ceratocystis ulmi or (Ceratostomella)  
Control: Chemical - Biological - Silvicultural  
Development Stage: Field Trial

The first year's work on the federally funded DED demonstration control project was completed. Yuma, Eaton, LaJunta and Canon City received intensive detection surveys, sampling, sanitation and insecticide preventive spray. (Michael E. Schomaker, Colorado State Forest Service.)

- B. Blue Stain  
Host: Pinus sp.  
Causal Organisms: Ceratocystis sp.  
Control: Chemical  
Development Stage: In Vitro

The herbicide paraquat exhibits differential growth inhibition of pure cultures of Ceratocystis sp. vectored by Dendroctonus sp. (F. Kidd, Col. State Univ. - Dept. Forest & Wood Sci.)

#### IV. ROOT DISEASES

- A. Root & Butt Rots in Managed Lands  
Host: Western hemlock  
Causal Organisms: Fomes annosus  
Control: Chemical - Silvicultural  
Development Stage: Impact Evaluation

Dissecting 400 trees in 20 rotation aged stands (half thinned half unthinned) to determine if losses being covered by root & butt fungi justify use of existing control measures. (D. Goheen, J. Hadfield, G. Filip, Region 6, Portland.)

- B. Shoestring root rot in plantations  
Host: Douglas-fir  
Causal Organisms: Armillaria mellea  
Control: Silvicultural  
Development Stage: Pilot Operational

An evaluation to determine if thinning heavily infected young stands in plantations decreases susceptibility of leave trees by increasing vigor. (G. Filip, Region 6, Portland.)

- C. Armillaria  
Host: Ponderosa pine  
Causal Organisms: Armillaria mellea  
Control: Chemical  
Development Stage: Field Trial

Plots established to test chemical control of Armillaria by treating around or in stumps. It's to be an expansion of Filip's and Roth's work on a little larger scale. A long term project beginning the fall of 1978. (K. Russell, WDNR, Olympia.)

- D. Armillaria  
Host: Pinus ponderosa, Abies grandis  
Causal Organisms: Armillaria mellea  
Control: Silvicultural  
Development Stage: Full Operational

A Manager's field guide with 40 colored pictures has been completed using principles set fourth by Shaw, Roth and others. Since each copy costs about \$40 it is not available for wide distribution with pictures. Text is available free and pictures by special arrangement. (K. Russell, WDNR, Olympia.)

- E. F. annosa  
Host: Western hemlock  
Causal Organisms: F. annosa  
Control: Silvicultural  
Development Stage: Field Trial

Annosus Root Rot in Precommercial thinning of Western hemlock, time of cutting and borax stump treatment 11 years after cutting = little control under conditions tested. (Driver, Edmonds, Chevez, UW Coll. of For. Res.)

- F. P. weirii  
Host: Douglas-fir  
Causal Organisms: P. weirii  
Control: Biological  
Development Stage: Greenhouse - Pot Trial

Greenhouse screening test of 2-0 Douglas-fir stock show presence of relative degrees of natural resistance to infection by P. weirii. (Driver, Edmonds & Morse, UW Coll. of For. Res.)

- G. Laminated root rot  
Host: Douglas-fir  
Causal Organisms: P. weirii  
Control: Silvicultural  
Development Stage: Field Trial

We are in process of laying out a field test of commercial thinning options in heavily infected Douglas-fir stands. (E. M. Hansen, OSU.)

- H. Fomes annosus root disease  
Host: Western hemlock  
Causal Organisms: F. annosus  
Control: Chemical  
Development Stage: Field Trial

In a field trial, application of granular zinc sulphate to freshly cut hemlock stumps prevented infection by F. annosus spores. (D. J. Morrison, Canadian Forestry Service, Victoria, BC.)

## V. DECAYS

- A. Decays  
Host: Douglas-fir, true firs  
Causal Organisms: Echinodontium tinctorium  
Control: Silvicultural  
Development Stage: Field Trial

Little decay in Douglas-firs wounded during thinnings in second-growth stands. Now studying Indian paint in white and grand fir advanced regeneration. (P. E. Aho, FSL, PNW Station, Corvallis.)

## BUSINESS MEETING MINUTES

The business meeting of the 26th Western International Forest Disease Work Conference was called to order by Chairman Dick Smith at 3:45 pm, October 27, 1978.

The minutes and treasurer's report were approved as presented in the Proceedings of the 25th WIFDWC.

### Old Business and Committee Reports

Dwarf Mistletoe Committee: Met for lunch on October 25, 1978. The committee report is included in the Proceedings.

Root Disease Committee: Walt Thies, Acting Chairman, summarized the topics discussed by the committee at the luncheon on October 25, 1978. Greg Filip will be chairman for 1979. Items discussed by the committee are summarized by Walt in the Proceedings.

Disease Control Committee: Ken Russell indicated that the committee discussed having a panel for the identification of needle diseases of the West. This would include a clinic or lab and would be held at a future WIFDWC. The committee is sponsoring the compilation of a national list of forest disease control methods presently in use; this will be coordinated by Dan Brown.

Interim Program Chairman: Dave Johnson's report suggests several possible discussion topics for future meetings. The most frequent comment made concerned the time available for discussion. Many people seemed to feel that there was not adequate time available to ask questions and discuss presentations. They felt that this should be a major consideration in the planning of future conferences.

### New Business

1980 WIFDWC location: Invitations were extended to the group by Neil Martin and John Laut to meet at Moscow, Idaho, and the Colorado State University, Henry Park Campus, respectively. After the advantages and disadvantages of each of the two locations were discussed, a show of hands revealed a preference for the Henry Park Campus as the location for the 1980 meeting.

Ed Wicker requested that someone else be appointed to keep the list of projects current. Frank Hawksworth consented to pick up this task. WIFDWC members want to thank Ed for seeing to this responsibility for so long.

### Election of Officers

Tom Laurent and Tom Hinds were chosen chairman and secretary-treasurer respectively for the 1979 WIFDWC. This was accomplished in the usual efficient and time-conserving manner.

The meeting was adjourned at approximately 4:15 pm.

TREASURER'S REPORT

		<u>Balance</u>
<u>Balance - carried over from 25th WIFDWC</u>		159.97
9-8-78	Dividend	2.42
		162.39
<u>Expenses Prior to 26th WIFDWC</u>		
10-18-78	Advance for Buses	100.00
		62.39
<u>Receipts - 26th WIFDWC</u>		
10-24 to 10-28-78	Registration	2375.00
		2437.39
<u>Expenses - 26th WIFDWC</u>		
10-24 to 10-28-78	Arizona Inn (including banquet, coffee, field trip lunches, etc.)	1790.90
	Buses (total less advance)	245.60
	Audio-visual equipment rental	47.00
	Misc. expenses and tips	39.10
	<u>Total Expenses</u>	2122.60
		314.79
<u>Receipts Subsequent to 26th WIFDWC</u>		
12-31-78	Dividend	2.24
		317.03
3-31-79	Dividend	4.92
		321.95
6-30-79	Dividend	5.00
		<u>326.95</u>
<u>Balance - June 30, 1979</u>		326.95

Deposit held: Washington State Employees' Credit Union  
P.O. Box WSECU  
Olympia, Washington 98507  
Account No. 936258

## INTERIM PROGRAM CHAIRMAN'S REPORT

David W. Johnson

The following topics were suggested for the 1979 meeting in Salem, Oregon:

1. A panel on disease problems in the Pacific Northwest and Alaska including aspen diseases (Hinds), hemlock dwarf mistletoe (Shaw). Suggested by Filip.
2. A panel on measuring impact due to forest diseases - growth loss, particularly loss due to root diseases. Suggested by Thies.
3. A panel on the specificity of non-specific diseases, including Verticicladiella wagnerii, Fomes annosus, and Armillaria mellea, all of which are non-specific in their action on conifers; however, they often attack only one or a few possible host species in a given area. The discussion should include recommendations for "replacement" species in diseases sites. Suggested by Parmeter.
4. A panel on foliage diseases and current control measures. Movie also available. Suggested by Staley.
5. Intensive forest management in western hemlock stands and the effects of Fomes annosus and hemlock dwarf mistletoe. Suggested by Hadfield (could be placed in panel as suggested in item 1).
6. Decay potential in understory true fir stands. Suggested by Hadfield.
7. The selling of "CODIT" in the Pacific Northwest. Suggested by Hadfield.
8. Diseases of seeds. Suggested by Hadfield.
9. The role of FI&DM (Forest Insect and Disease Management). Suggested by Don Graham.
10. A panel on the status of the Dutch elm disease demonstration projects, including Lake States, Colorado, and California. Dan Brown volunteered to lead panel.
11. New developments in white pine blister rust programs, emphasizing silvicultural control. Suggested by Byler.
12. A discussion on current fungus name changes and reasons for. Suggested Gilbertson lead discussion. Suggested by Hal Bursall (Forest Products Lab).

**Other program suggestions:**

Allow more time for discussions of new projects and techniques. Rather than list projects, give some detailed information.

Have "time" available for discussion of current problems, identification of specific pests. A "show and tell" session.

T-shirts with some "catchy" slogans were present at this last meeting. A suggestion was made that we offer an award (T-shirt award) for the best slogan at the banquet.

Have questions for speakers on panels immediately following presentations rather than delay questions until end of panel.

Panels should be organized to present a central theme or idea rather than a potpourri of unrelated subjects. This last meeting was characterized by too many unrelated subjects and paper presentations. It would be much better to reduce the number of papers and panels and allow for more discussion.

## NEW PUBLICATIONS

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- Aho, P.E. and L.F. Roth. 1978. Defect estimation for white fir in the Rogue River National Forest. USDA For. Serv. Res. Pap. PNW-240, Pac. NW For. and Range Exp. Stn., Portland, OR. 18 pp.
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- Crawford, D.J. and F.G. Hawksworth. 1978. Flavonoids of the genus Arceuthobium (Viscaceae). Bot. Soc. Amer. Misc. Publ. 156:8 (Abstr.).
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