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Proceedings of the Twelfth Western International Forest Disease Work Conference
Berkeley, California

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FOREWORD

The twelfth Western International Forest Disease Work Conference was held October 12-16, 1964, in Berkeley, California. Both the Pacific Southwest Forest and Range Experiment Station and the Department of Plant Pathology, University of California, were hosts for the conference.

Seventy-four members and guests registered for the conference. Registration was held in the Pacific Southwest Station Conference Room, Monday morning. In the afternoon refreshments were served and the conference members were welcomed to the Station by Director John McGuire. The remainder of the afternoon was spent visiting the University and Experiment Station laboratories and in informal discussions among host and visiting members.

The meetings began Tuesday morning and were held in the Regents Room, University Hall, University of California. Chairman Shea opened with his welcoming remarks and Dr. W. C. Snyder, Department of Plant Pathology, welcomed the members to Berkeley on behalf of the University of California. Three days of stimulating meetings followed. On Friday a field trip was made to Boggs Mt. State Forest via the Napa Valley, a noted wine-growing region. Those making the trip not only were exposed to gall rust, Fomes annosus, and dwarfmistletoe, but were also able to visit the wineries and sample some of California's fine wines.

The banquet, held Wednesday evening at Spenger's Fish Grotto, was attended by 87 members and guests. Reed Miller, on his own home ground, won the annual social achievement award not only for his brilliant performance at this year's banquet but also for his sustained, outstanding efforts over the past years. Entertainment at the banquet was provided spontaneously by the greater part of the attending members and guests.

The business meeting was held on Wednesday afternoon and adjourned on time in favor of the previously scheduled and long anticipated evening's activities.

Concluding remarks by Chairman Shea, the Secretary-Treasurer's report, and adjournment of the 12th WIFDWC took place on Thursday afternoon.

Executive Committee
Keith Shea, Chairman
Robert F. Scharpf, Secretary-Treasurer

Program Committee
C. D. Leaphart, Chairman
H. R. Offord
A. Molnar
S. Whitney

Local Arrangements
H. R. Offord
J. R. Parmeter
A. H. McCain
R. V. Bega
H. H. Bynum
D. R. Miller

OPENING REMARKS

Chairman Keith Shea

Ladies and Gentlemen:

Today marks the beginning of the 12th W.I.F.D.W.C. Through these 12 years this conference has attained widespread recognition as being the best of its kind. This has not occurred by chance but is the result of active and enthusiastic participation of all members in an atmosphere conducive to free and informal exchange of ideas.

For those of you who have not previously attended this conference we extend a special welcome. We hope this will be a rewarding and unique experience. Feel free to voice your queries and opinions. It is our custom to dispense with formality, to advance our philosophies with vigor, and to discuss our differences without rancor. We want your constructive thoughts and want you to feel among friends.

Your program chairman, Charles Leaphart, and his committee, Toby Childs, Alex Molnar and Harold Offord, have laid a firm foundation for forward-thinking and thought-provoking discussion.

Your local arrangement committee consisting of Harold Offord, chairman, Art McCain, Dick Parmeter, Bob Bega, Hart Bynum, Bob Callaham, Bob Scharpf, and Reed Miller has arranged for the framework in which we can discuss the presentations as well as mutual problems.

It is now up to us, by our participation and discussion to complete this structure which has been planned for us. We may, perhaps, be ready to raise the roof at our annual banquet!

I would like now to call upon our honored guest, Dr. W. C. Snyder, Chairman, Department of Plant Pathology, University of California, to deliver the welcoming address.

PANEL I - ASPECTS OF BIOLOGICAL CONTROL OF TREE DISEASES.

A. C. Molnar - Moderator

BIOLOGICAL CONTROL OF FOREST TREE RUST AND NEEDLE-CAST PATHOGENS

Paul D. Keener

The development of biological methods for the prevention or suppression of rust and needle-cast pathogens attacking forest trees and their associated vegetation (collectively regarded as wildland plants) should have numerous advantages. The unique and changing environments accompanying multiple uses for vast acreages of forest in which disease-inducing organisms proliferated, afford an excellent field for investigations into heretofore non-existent biotic systems. Biological control of forest tree pathogens should result in fewer hazards to users of recreational and other areas. Water, one of the chief products of watersheds, should be less if at all contaminated, if biological methods are substituted for chemical. Fish and wildlife will be better protected against toxicities. Biological methods might prove more effective against obligate parasites such as viruses and rusts as well as needle-casts, all of which are pathogens difficult to control by the customary synthetic chemicals.

An extensive discussion about methods used in attempts to control rust and needle-cast pathogens biologically is impossible for obvious reasons and is not intended here. Some techniques which have been, or are currently in use, are briefly reviewed.

(a) Eradication of alternate hosts of heteroecious rusts either by physical or chemical methods, is one of the classical "textbook approaches" for reducing losses from such pathogens as Cronartium ribicola Fisch., the cause of White Pine Blister Rust. Eradication, at least somewhat effective in controlling obligatorily heteroecious species, might prove fruitless in efforts to combat possible "facultative heteroecious" rusts, if the latter do exist in nature. Artificial inoculations by Molnar and Sivak (20) demonstrating the susceptibility to infection by Melampsora albertensis Arth., of seedlings of coniferous genera and species other than Pseudotsuga spp., the usual natural host for the pycnial and aecial stages, could forewarn of some of the complications likely to be encountered in attempts to devise biological methods for the control of heteroecious

rusts. Perhaps field infections of seedlings of coniferous genera other than Pseudotsuga occur, but the plants are killed before there is any chance to observe them.

Seedling mortality accompanied by poor natural regeneration for Pinus ponderosa Laws., has been noted on the Coronado National Forest, Santa Catalina Mountains, near Tucson, Pima County, Arizona. This has been assumed to be due to the usual cycling of Cronartium coleosporioides Arth. (sensu Peridermium filamentosum Pk.) between the Pine Castilleja spp. even though as later pointed out, Castilleja and other susceptible scrophulariaceous plants are gradually decreasing in the area. Melampsora albertensis on leaves of Populus tremuloides Michx. var. aurea (Tidestrom) Daniels is quite prevalent, yet needles on the expected alternate host in the area (Pseudotsuga Menziesii (Mirb.) Franco (Douglas-fir)) do not show infections. The anomalous life cycles of certain heteroecious rusts, in which one or more stages are eliminated, might be due to adaptation to "facultative heteroecism."

Greater rust-susceptibility of seedlings in contrast to that of older tissues has been reported by Patton (21). Seedlings of Pinus strobus L. were more susceptible to Cronartium ribicola Fisch. than grafts.

The distances to which rust and needle-cast spores will spread and still retain viability and infective capacities has been determined for a number of forest pathogens. This is an important consideration in evaluating attempts to control them by biological agents.

(b) A recent approach to the problem of biological control of forest tree pathogens, embodying an emphasis on rust and needle-cast fungi, has been in the use of chemotherapeutants including some of the antibiotics and their derivatives. This subject is to be discussed by participants in Panel II. It is only emphasized here that experiments aimed at the possible biological control of Cronartium ribicola are in progress. Rhabdocline pseudotsugae Syd. (the cause of Rhabdocline needle-cast of Douglas-fir) has also been the subject of similar studies (27).

Some of the distinct advantages in the use of such preparations against widely distributed pathogens such as those attacking forest trees, are: Effectiveness at low concentrations, small amounts-per-acre required, and low costs of applications. Glew and MacLeod (6) offer some interesting data on this matter.

Since recreational and watershed areas may require concentrated

attention with respect to the control of forest tree pathogens, the successful use of antibiotics and their derivatives, would be particularly appealing.

A review on the subject of the use of antibiotics against plant pathogens has been published by Dekker (5).

(c) Genetic manipulations resulting in the development of clonal lines of forest trees tolerant or resistant to attacks by pathogens, are of comparatively recent adoption. Methods for the rapid increase of promising lines by selection, asexual propagation and other devices, should provide a reservoir of elite stock in the future. Some results have already been reported with respect to the development of lines having some tolerance or resistance to Cronartium ribicola, Fusiform rust and others. In Arizona, there are indications of tolerance to needle-cast pathogens by individual specimens of Pinus ponderosa Laws.

Some of the genetic and related problems likely to be encountered in developing disease-tolerant or resistant trees, have been discussed by Riker and Patton (22).

(d) Silvicultural practices designed to improve growth, quality, and other characteristics of disease-tolerant or resistant stocks, certainly comprise a part of the concept of disease pathogen control by biological methods. Improvement of sites as well as other silvicultural practices often impede the rapidity of spread if not actually prevent the dissemination of forest pathogens. Wider spacing of pines in mixed stands will aid in reducing amounts of infection by Cronartium ribicola according to Cafley (3). The location of Ribes spp. near stands of susceptible pines is to be avoided.

Burnings for the control of the pathogen causing Brown Spot Needleblight, Scirrhia acicola (Dearn.) Siggers on Pinus palustris Mill. are a form of indirect biological control. In these instances the fungus is controlled without the complete eradication of the suscept.

(e) Predator attacks on rust spores and other structures have been reported. (9, 19). The literature indicates that both mites and insects have been implicated. The reduction in inoculum by such predators is probably insignificant.

(f) The natural occurrence of numerous non-uredinaceous microorganisms (particularly fungi) antagonistic to rusts has been the subject of numerous investigations as to the possible use of such agents in the biological control of rust pathogens. Techniques have been devised to inoculate assumedly "hyper-parasitic" fungi unrelated to, but with the capacity to colonize various types of rust sori.

The term "hyperparasitism" has been applied to the simultaneous association of the unrelated microorganisms colonizing the rust sorus. The validity of the terminology "hyperparasitism" for all the reported associations is questionable.

No data are known concerning the use of "hyperparasites" in attempts to control needle-cast and needle-blight pathogens of forest trees. Recent studies (11) disclose instances of simultaneous cohabitation of fruiting structures of both needle-cast and needle-blight pathogens with unrelated fungi. This phenomenon has been observed in species of the genera; Elytroderma, Hypodermella and Hemiphacidium. In fruiting structures of Hypodermella medusa Dearn., and an apparently undescribed needle-cast pathogen of the same genus, cases of "double hyperparasitism" have been observed, in which more than one unrelated fungus occurs simultaneously.

In nature, symbioses between two or more unrelated microorganisms are commonplace. The physiological bases for these associations are not so obvious. It has been assumed, unsupported by experimental evidence, that these associations represent phases of symbioses from parasitism, commensalism, mutualism through merely the fortuitous occurrence of the participants. The role of these associations in the "balance of nature" is frequently not well understood.

The citation of numerous reports in the literature concerning only those associations involving rusts and unrelated fungi would have no value in the ensuing discussion. Certain features concerning some of these associations should be examined.

Long before modern chemotherapeutants including antibiotics and their derivatives became available, scientists were examining possible techniques for the biological control of forest tree pathogens. As early as 1914 and later, von Tubeuf (25, 26) published the results of experiments designed to control Cronartium ribicola Fisch. and other species of this genus, by artificially inoculating aecial cankers on Pinus spp., with the non-uredinaceous hyperparasite, Tuberculina maxima Rostr. The reported success of these experiments formed the basis for von Tubeuf's claim of "Biologische Bekämpfung" (25, 26). Previous to von Tubeuf's reported successes in controlling Cronartium ribicola and others with the now recognized hyperparasite Tuberculina maxima, Sappin-Trouffy (23) germinated conidia of Darluca filum (Biv.) Cast. (Eudarluca) in contact with spores of Carnation Rust (Uromyces dianthi (Pers.) Niessl) on the surface of water cultures in the laboratory. The germ tubes of the conidia invaded both the uredio- and teliospores of the rust. Sappin-Trouffy's results were apparently the first experimental demonstration of rust spore attack by a non-uredinaceous fungus. Other investigators such as Goodding (7) and Hubert (8, 9) reported on the occurrence of various fungi colonizing rust sori in the field as well as on their attempts at biological control of the pathogens. Hubert (9) referring to European successes in controlling pathogens biologically, admits his apparent failures with Tuberculina maxima on aecial cankers of Cronartium ribicola on Pinus in Idaho.

That contact between conidia and rust spores is not a necessary condition for the growth and reproduction of Darluca (Eudarluca) filum was experimentally demonstrated by Keener (12) in 1933. D. filum from 10 different rusts was isolated into pure cultures on several synthetic media. Both conidia and ascospores (Eudarluca) served as units of isolations (13, 14). Although all of the rusts from which isolations of the

hyperparasite were made were non-forest tree pathogens, this was the first demonstration that D. filum would grow and sporulate apart from the normal uredinaceous host. This phenomenon has not yet been demonstrated for the rusts themselves. It was also shown that growth of the hyperparasite on various media indicated the existence of 6 groups of isolates derived from the 10 rusts (12). The absence of cultural similarities indicating some degree of physiological specialization was further verified in greenhouse cultures of D. filum, both on the same species of rusts from which they were originally isolated and on "newly challenged suscepts" (13, 14). Previous to this experimental evidence, symbioses of non-uredinaceous fungi and rusts, were regarded primarily as obligate parasite-obligate parasite systems. It is now evident that in some cases the physiological relations simulate facultative parasites with obligate parasites. The ease of isolation and subsequent copious sporulation of D. filum on artificial synthetic media, provided ample inoculum for studies on the possibilities of utilizing a non-uredinaceous fungus for the biological control of a rust. Results of these studies have already been reported (14). In general, it was concluded that D. filum does not display the same specificity for rusts as do the latter for their phanerogamic and cryptogamic suscepts. In no instance, was significant biological control of the rusts obtained even with controlled environmental conditions. The hyperparasite attacked and destroyed rust spores, thereby reducing the amounts of inocula available for additional infections.

A comprehensive listing of the numerous rusts and their non-uredinaceous colonizing microorganisms apparently has never been attempted. The review by Schroeder and Hassebrauk (24) mentions 35 genera and numerous species of unrelated fungi reportedly capable of colonizing rust sori. This review also confirms the successful isolation and subsequent growth of D. filum on artificial synthetic media. Other reviews with speculations as to the possible uses of non-uredinaceous fungi in the biological control of rusts, have been published by Hulea (10), Kirulis (17) and Keener (13, 14).

It is evident from the preceding and ensuing data that all types of rust sori produced by micro- and macro-cyclic, as well as autoecious and heteroecious rusts, are susceptible to colonization by unrelated, non-uredinaceous microorganisms, particularly fungi. Certain species such as Darluca, Tuberculina and Verticillium appear capable of destroying rust spores, while others like Cladosporium apparently lack this capacity and probably only impede the dissemination of these pathogens. Barnett (2) states that Darluca filum is highly specific as a parasite on rust mycelium, but omits any mention of spore destruction. Since the physiological phenomena associated with the action of the hyperparasite on rust structures has never been ascertained, particularly with respect to nutritional balance in favor of one or the other partners, it is perhaps preferable to consider such "hyperparasitism" merely as associations of fungicolous fungi.

In heteroecious rusts, the specific non-uredinaceous fungi associated with the sori and spores on one of the hosts may not be

the same as those colonizing similar structures on the botanically unrelated alternate plants. Hence, with any particular species of heteroecious rust, the microorganisms invading the pycnial (0) and aecial (I) stages may be quite different from those inhabiting the uredinial (II) and telial (III) sori. Also, two different non-uredinaceous microorganisms have been observed colonizing individual sori of the same type, simultaneously (15). Cases have been observed in which different species of colonizing fungi occupied individually sori of the same type produced by the same rust on the same leaf (unpublished data).

Although Cladosporium aecidiicola is the most frequently encountered fungus in all types of rust sori and possibly has been so in the past at least on certain needle-infecting Uredinales, the potential efficiency of this species in the biological control of rusts has never been evaluated.

In the Southwestern United States, mountainous outcroppings from the desert valley floors, all part of the western slope of the Rockies, support wildland vegetation of high economic value with respect to watershed and recreational uses. In southeastern Arizona, the heteroecious rust, Cronartium conigenum Hedgc. & Hunt alternates between Pinus leiophylla Schiede & Deppe var. chihuahuana (Engelm.) Shaw (0 and I stages on cones = conicolous) and various species of Quercus, especially Q. hypoleucoides Camus (II and III stages on leaves = foliicolous). Both Fusaria and Cladosporium spp. have been isolated from aecial sori but only after the cones have become enlarged, hardened and mummified. In spite of the accompanying destruction of seed suitable natural regeneration occurs. Examinations of 100 cones from randomly selected trees, over several week periods for the past three years, disclosed that topography apparently influences the amount of infection. Greater percentages of infected cones were found at higher elevations than at lower. On south slopes infections were more severe than on north. On the latter some trees had no infected cones. Figures indicated are for south slope trees only.

Tree locations	Total number of cones examined	Number of cones free from observable rust infection	Number of rust-infected cones	Percentages infected cones
In low elevations	1879	1840	39	9.7
On ridges & hillsides	2204	1935	269	13.1

When uredinial sori appear on smooth-leaved Quercus spp., infections are likely to be so severe as to render an entire tree orange-colored. Darluca filum invades the uredinial sori. D. filum has not been observed in aecial sori on rusted cones. Conversely, Fusaria and Cladosporium spp. have not been seen on uredinial or telial sori on rusted Quercus leaves.

Although "facultative heteroecism" is apparently not involved here, the disease appears to have become static, with no indications of further extension. In view of the huge amounts of uredinial inoculum and the expected build up by such repeating spores, it could be that Darluca filum is the agent responsible for the static nature of the disease. Since Quercus leaves with uredinial sori and Darluca are understory and thus quite removed from the cones on pine, the hyperparasite apparently does not come into contact with the aecial sori.

The foregoing data illustrate some of the biotic responses among fungi colonizing sori of alternate stages of a heteroecious rust, when the cycle of the latter is between two unrelated genera of forest trees.

There is also stabilization and absence of extension in local areas of the Southwest, in which Cronartium coleosporioides Arth. (sensu Peridermium filamentosum Pk.) alternating between Pinus ponderosa Laws. (0 and I on branches = caulicolous) and certain scrophulariaceous plants (Castilleja spp. and others with II and III on stems and leaves = caulicolous and foliicolous). While some of this phenomenon has been attributed partially to anomalous life cycles, in which certain spore stages of the rust fail to develop fully or are eliminated, other causes might be involved. No hyperparasitic or non-hyperparasitic microorganisms have ever been noted in any of the sori of this rust. The scrophulariaceous species serving as alternate hosts for the uredinial and telial stages, are gradually disappearing from areas even where the rust infection is severe. This could be due to a "self-imposed limitation" on the part of the rust. It is possible that the aeciospores have developed the capacity to re-infect pines directly, in which case anomaly of life cycle would be involved. Our studies reveal almost negligible extension of the aecial stage of the rust on Pinus ponderosa over the past 8 years from a heavily invaded area on the Coronado National Forest, Santa Catalina Mountains, near Tucson, Pima County, Arizona.

Gradual disappearance of proximal alternate vegetation accompanied by stabilization in the extension of the rust, has been noted with Gymnosporangium cupressi Long & Goodding. The pathogen supposedly alternates from Cupressus arizonica Greene on which only the telial stage is produced and species of Amelanchier harboring the pycnial and aecial stages. Amelanchier has apparently been heavily browsed by wildlife and has virtually disappeared from an area in which the rust has caused severe cankering on Cupressus trunks.

Not too surprising is the continued severity of infections of needles of Pinus edulis Engelm., by the autoecious micro-cyclic rust, Coleosporium crowellii Cummins, with the telial stage only on this single species. Both Darluca filum and Cladosporium aecidiicola occur on the telial sori. Some details concerning the colonization of telial sori have already been described (16). Hyperparasitism of telial sori without an intervening uredinial stage, occurs in heteroecius rusts such as in species of Gymnosporangium. In all of these instances, although hyperparasitism is acting through Darluca filum, in addition to fortuitous colonization by Cladosporium spp. no diminution in the density of plants supporting autoecious or heteroecious rusts has ever been noted. The intensity of colonization by non-uredinaceous fungi continues at high levels year-after-year as do the rust infections. No apparent biological control occurs. Extensions in infections occur. On the other hand, extensions of heteroecious rusts with uredinial stages on alternate hosts in their cycles, appear to be limited in their extensions from year to year.

Langner (18) describes an interesting biotic system in which there is an apparent relation between a preinfection of pine needles by Phoma acicola (Lév.) Sacc. and later development by the needle-cast pathogen, Lophodermium pinastri (Schrad. ex Fr.) Chev. Some method of biological control of the former should prevent the needle-casting from trees.

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Appendix - 1

NEW OR UNUSUAL RECORDS ON FUNGI COLONIZING SORI OF RUSTS ATTACKING SOUTHWESTERN WILDLAND PLANTS

Rusts	Hosts 0 + I stages	Colonizing fungi (Genus only)	Hosts II + III stages	Colonizing fungi (Genus only)
Chyrosomyxa arctostaphyli Diet.	Picea spp.	Cladosporium	? Arctostaphylos spp.	
Coleosporium crowellii Cummins	None		III stage only Pinus edulis	Cladosporium Darluca
Coleosporium jonesii (Peck) Arth.	Pinus edulis	Darluca phoma	Ribes spp.	Cladosporium Darluca
Cronartium conigenum Hedgc. & Hunt	Pinus leiophylla var. chihuahuana	Cladosporium Fusarium	Quercus spp.	Darluca
Gymnosporangium kernianum Bethel	Amelanchier spp.		III stage only Juniperus spp.	Darluca
Gymnosporangium multiporum Kern	Lacking or unknown		III stage only Juniperus spp.	Darluca
Ravenelia versatilis Diet.	Acacia greggii	(Autoecious)	Acacia greggii	Darluca

NEW OR UNUSUAL RECORDS OF FUNGI ASSOCIATED WITH OR COLONIZING FRUITING BODIES OF SOUTHWESTERN NEEDLE-CAST PATHOGENS

Needle-cast pathogen	Host	Associated or colonizing fungi
Hypodermella abietis-concoloris (Mayr) Dearness	Abies concolor	Macrophoma parca
Hypodermella concolor (Arizona variant)	Pinus ponderosa	Davisiella-Diplodia complex Phaeoseptoria Phoma
Hypodermella medusa Dearn.	Pinus ponderosa	Davisiella-Diplodia complex Phaeoseptoria Phacidium (Hemiphacidium) spp.

MICROBIOLOGICAL COMMUNITIES AND THE POSSIBILITY OF BIOLOGICAL CONTROL OF TREE DISEASES

Gertrude D. Pentland and John E. Bier

At the Work Conference in Victoria in 1962, Dr. Bier (1962) introduced us to the idea that healthy plant tissues should be considered not as entities but as complex biological communities, consisting of host tissues plus numerous saprophytic organisms. In the consideration of a disease problem the incidence and activities of these saprophytes may prove of critical importance to the degree of host resistance and the development of the pathogen. With this concept in mind, Dr. Bier and his students have worked on several different types of diseases in the last few years. I shall summarize briefly the results of these experiments and discuss some of the implications and technique difficulties. I should like to point out that very little of the material which I am presenting is work that I have done.

Experiments have been carried out on different types of diseases, using the organisms and hosts as shown in the following table: (see accompanying chart - it will be shown as a slide).

A general outline of the methods used includes first of all the selection of apparently healthy host material. The isolation of saprophytes from it has usually consisted of shaking pieces of the host material - wood, bark, leaves, etc. - in sterile distilled water for a period of from one to several hours. This method has the advantage over the standard technique of placing small pieces of plant material on agar in that one can isolate a number of different organisms from one sample, obtaining at the same time some idea of the range of organisms present. Also the same sample of shake liquid (suspension) can easily be added to a number of different media suitable for different types of organisms.

To test for the effect on disease development of the saprophyte isolated by shaking, the treatments are as follows:

1. sterilized host material alone (control)
2. sterilized host material + suspension of saprophytes
3. sterilized host material + Millipore-filtrate of the suspension.

The treatments of host samples to test for the effect of the naturally-occurring community on disease development are as follows:

1. untreated (i.e. containing the natural community)
2. surface-sterilized by dipping in alcohol and/or flaming
3. autoclaved or dry-heat sterilized

TYPE OF DISEASE	ORGANISM	HOST
Canker	Hypoxylon Pruinatum	Populus Tremuloides
Wood Rot	Stereum Sanguinolentum	Abies Lasiocarpa
	Poria Monticola	Pseudotsuga Menziesii
		Tsuga Heterophylla
	Fomes Annosus	Tsuga Heterophylla
Rust	Melampsora Occidentalis	Populus Trichocarpa
	Cronartium Harknessii	Pinus Contorta

Special treatments to test the effect of different factors such as relative turgidity can be included within this general outline. The samples are inoculated (the type of inoculum is of considerable importance because of nutritional effect of the inoculum medium on the saprophytes) and variations in the development of the pathogen and of the disease are noted. This general procedure is modified, of course, for different types of disease and different host parts.

The results of the studies carried out at U.B.C. will be presented as a theme with variations. The theme, which should be stated clearly at the beginning, is that there appears to be a relationship between the intensity of saprophytes in and on the host tissue, the turgor level of the healthy tissues and the level of host resistance to disease. That is, the microbiological community plays an important role along with turgor in determining susceptibility to disease.

As an example of a canker disease, Bier and Rowat have worked with Hypoxylon pruinatum on Populus trichocarpa and P. tremuloides. They have demonstrated (1962) that protection against Hypoxylon disease was at a maximum when host tissues contained an abundant supply of water (which was consistent with the bark turgor relationship previously demonstrated, Bier, 1961). Removal of the saprophytes from the host resulted in disease susceptibility at all levels of moisture investigated. It appeared that the saprophytes had a higher moisture requirement for optimum development than that of the pathogen. Canker attach by H. pruinatum was delayed considerably when drying, viable material of one clone of P. trichocarpa was dipped in a suspension containing an abundance of the saprophytes prior to inoculation. Bier and Rowat (1963) have also shown that certain saprophytes associated with healthy bark tissues may inactivate chemical inhibitors

such as pyrocatechol. However the saprophytic microfloras of most samples contained other organisms such as Trichoderma sp. and Aspergillus sp. which were mutually antagonistic with H. pruinatum. The growth of the pathogen was inhibited when untreated bark tissues were colonized by these saprophytes.

As indicated earlier, studies have been carried out on the effect of microbiological communities on several wood-rotting fungi. Bier (unpublished) has demonstrated that saprophytic microorganisms occurred in decay-free heartwood of Abies lasiocarpa, some of which were highly effective in inhibiting the growth of Stereum sanguinolentum in culture and, when present, appeared to increase the resistance of heartwood to attack by this fungus. The composition of the microflora was variable in samples within and between trees. The inhibiting saprophytes were most effective in preventing the growth of S. sanguinolentum when the moisture content of the heartwood was increased to values greater than 90% of its oven-dry weight. Excellent growth of Stereum occurred in over-dried samples of heartwood at all levels of moisture investigated. The growth of the fungus was stimulated rather than inhibited when Millipore-filtrates of suspensions containing the saprophytes were added to oven-sterilized sawdust of heartwood tissues. Therefore, it appeared that the inhibition of Stereum was more closely related to the biological than chemical component of the suspensions.

A study was set up by Bart van der Kamp (1964) to determine the relative importance of Douglas-fir heartwood extractives and heartwood microflora in providing resistance to decay by Poria monticola. Results showed that while sterile heartwood was more resistant to decay than sterile sapwood, the difference was completely masked by the addition of saprophytes. Saprophytes in wet sapwood provided more resistance to decay than heartwood extractives in sterile wet heartwood. There was some evidence that the inhibiting effect of the saprophytes on P. monticola was produced by a combination of the saprophytes present. Single saprophytes produced little resistance to decay.

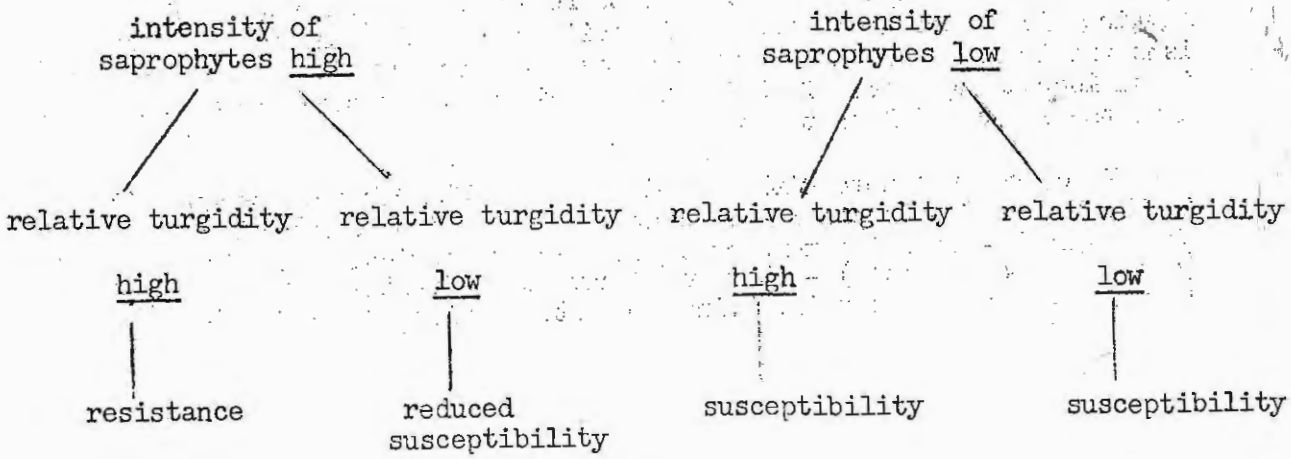
Working with western hemlock, Janos Hudak (1964) demonstrated that the saprophytes isolated from apparently sound wood inhibited the growth of Fomes annosus and Poria monticola when added to 1% malt agar. The decay fungi were also inhibited on untreated surface-sterilized wood incubated at 100% relative humidity. A drier environment, obtained by using a lower R.H. for wood and higher agar concentration in malt agar media, resulted in a significant decrease in inhibition of F. annosus and P. monticola. However, the growth rate of these organisms was not affected in autoclaved wood or in agar containing the autoclaved suspensions in the drier conditions. Significant variation was observed in the rate of inhibition between sapwood and heartwood and between the butt and top sections of the trees. The inhibition was lost when the wood was autoclaved or when the suspensions of saprophytes were passed through a Millipore filter.

As an example of a rust disease Dr. Bier (unpublished) has studied uredineal infection of Melampsora occidentalis on Populus trichocarpa for several years. He first found that uredospores would germinate readily on water agar if they had been collected after a dry period, but germination was negligible after a wet period. Cultural studies of the non-germinating uredospores demonstrated heavy contamination with saprophytic organisms such as Trichoderma sp., Epicoccum sp., Aspergillus sp., and a number of bacteria and yeasts. Experiments using the suspensions of saprophytes from healthy foliage demonstrated that germination was reduced in the presence of the saprophytes to 0-69% of that in sterile water and in Millipore-filtrate controls. Experiments with cuttings showed resistance to infection when leaves were sprayed with an aqueous suspension containing the inhibiting saprophytes prior to inoculation. However, uredinia were produced abundantly on leaves of the same cuttings sprayed with the Millipore-filtrate.

Field trials with Melampsora rust have indicated that the incidence and intensity of different organisms on healthy foliage may be modified by applying aqueous suspensions that contain an abundance of inhibiting saprophytes. Such modifications may become well established and survive over a period of months, providing increased resistance to rust infection. The increased resistance of host material treated with inhibiting saprophytes may lessen as foliage reaches maturity because of a reduction in the activities of the protective saprophytes related to decreases in levels of leaf turgor. However, because of an abundance of the inhibiting saprophytes treated foliage may retain the resistance factor at lower levels of leaf turgor than untreated controls.

Studies are now under way on two more rust diseases, Cronartium harknessii on Pinus contorta and Melampsora albertensis on Pseudotsuga menziesii. The indications are that the situation is similar for these diseases, with the interesting addition that the bacterial members of the community seem to be particularly important here.

And now my theme can be restated, this time in the form of a chart. The situation seems to be as follows for all the diseases considered:



From the results presented, it appears that there is a community of microorganisms present as part of the normal total environment of a plant. There may be considerable variation in the community in terms of types, numbers and distribution of organisms within an individual plant and between plants of one species. However, it seems that the microbiological population must be considered as a factor in the normal pattern of disease resistance, on the same level as genetic resistance, physiological resistance, etc. The possibility that susceptibility results from a disturbance of the microbiological community should be taken into account along with a consideration of the other factors.

Of course there are many problems involved in this type of research and many areas needing more work. Little is known about the actual nature of the inhibition of a pathogen by a microflora under natural conditions. Individual organisms vary in their ability to inhibit pathogens, but it seems that several organisms together are more effective than any one alone. Is this an additive antibiotic effect or just a question of coverage? Perhaps there are individual "micro-niches" which are occupied by different organisms and so using several different organisms results in better coverage by having more "micro-niches" occupied. Why is there a relationship between inhibition and the turgor level of the tissues? And of course there are other physiological factors of both plant and saprophytes involved, of which we know very little.

What factors affect the location and distribution of microorganisms under natural conditions? In what state are they present? Are they active forms such as growing mycelium or are they just spores? Are they dependent on exuded nutrients? - perhaps that is a factor which is affected by the turgor level. Is the distribution purely by chance? The possibility that there are differences in the saprophytic microfloras related to differences in the soil microfloras which vary on different sites might be considered, because many of the organisms are also soil inhabitants.

If saprophytic organisms are added as an attempt at biological control will they become established and survive? It appears from the work done at U.B.C. so far that there is not the same type of fungistatic effect that there is in soil because the numbers of organisms and individuals is increased by adding suspensions. Also there is an increasing level of resistance to disease. We don't know how long the effect will last, though.

To close on a note with regard to biological control, because it is the topic of this panel - if control methods can be worked using the naturally-occurring members of the microbiological community - (and it seems possible) - they would have the advantage of being much less risky than methods involving the introduction of exotic organisms.

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BIOLOGICAL CONTROL OF DWARFMISTLETOES

Robert F. Scharpf

Biological control is not a unique new concept thought up by scientific minds but a natural phenomenon that occurs among all living things. The modern-day use of the term biological control includes the use of antagonists, parasites, and predators to control plant and animal pests. Environment, genetic resistance, and management are some of the interacting factors included in biological control.

Early pioneering studies on biological control dealt mainly with the control of destructive insects by insect pathogens and predators. However, since about the beginning of the Second World War there has been considerable interest and scientific investigation in the field of biological control of weeds.

Dwarfmistletoes are weeds. Thus, the principles and approaches that apply to the biological control of weeds probably in many instances can be applied to the control of dwarfmistletoes.

As in the case with many new scientific concepts, biological control of weeds was not readily accepted. According to Wilson (4), because of lack of understanding, scientific orthodoxy did much to hamper development and progress of the biological control of weeds, mainly by discouraging research.

One of the major objections voiced against biological control was based on the fear that introduced organisms, primarily insects, will become pests of other crops. Indeed, probably the first consideration in initiating a biological control program is to select a species that will not harm other plants, at least useful plants.

Just what are the chances a particular insect will change his diet and become a pest? According to Wilson, there is little foundation for the fear that insects will change their host ranges. Recent research stresses the stability of insect food habits, not the likelihood of change. Huffaker (1), who has examined records of changes in diet, has shown that most records concern oligophagous insects whose hosts have merely become better known. In general, it is the conclusion of most researchers in the field of biological control that the feeding habits of plant-feeding insects are very rigid and nearly unchangeable. Also, the logical assumption made by most researchers is that introduced organisms have no greater chance of undergoing inherent changes to new hosts than the numerous indigenous organisms have. It is understood, however, that organisms to be considered for biological control will be subjected to thorough testing before they are released in the field.

Conflicts of interest frequently arise when the biological control of a weed is considered. A plant may be a scourge in one place and of value in another, or in the same locale, it may be desired by one group or deplored by another. Unlike chemical control a biological control agent cannot easily be restricted to given parcels of land. Insects and disease organisms for example do not respect political boundaries or differences in land ownership. They move in where a host or a source of food is available.

Although few people would object to the control of dwarfmistletoes, other allied genera are of economic importance. Certainly from the recreational as well as the economical standpoint we would not want to perpetuate an organism that would eradicate the mistletoe we hang over the doorway each Christmas. As Huffaker (1) points out some of the factors to be considered before initiating a biological control program should include future values as well as present ones, minority as well as majority rights, interests of another states or nations, and the effects on other plants or animals.

Most of the objections to the biological control of weeds as previously mentioned have since been discounted or are at least understood and a change in attitude is taking place, stimulated primarily by the successes obtained against some major weeds and the fact that no insect introduced for weed control has become a pest.

So far most all of the discussion has been concerned with the biological control of weeds with insects. Let us now take an overall look at the possibilities of using disease organisms as biological control agents of weeds.

To begin with, what problems might be expected in using disease organisms as biological control agents. As I see it in most cases, a number of problems are involved. Certainly there can be conflicts of interest regardless of what type of biological control agent is used. And risks to economic plants are definitely involved when introduced diseases are considered for biological control.

In general, I might say that the basic problems confronting researchers in using diseases for biological control are much the same as those confronting researchers using insects as control agents, and that the problems of testing diseases for control are equally as difficult if not more so.

Is biological control of dwarfmistletoes possible? It seems to me that the possibility of developing a biological control through continued research is quite good. First let me say that control of this pest, as well as many others, to be economically sound need not be 100 percent effective. It is likely that a reduction of existing infection or reducing subsequent spread by even 50 percent would be considered excellent control. Of course the actual limit of eccnomic feasibility and benefits achieved from a biological control program

cannot at this time be estimated. These types of questions will have to be answered during and after the course of the research and control project.

What are the possible biological control approaches that may be taken to reduce damage from dwarfmistletoes? In general, two broad approaches may be taken. The first is aimed at eliminating existing infection; the second aimed at preventing further spread of the disease (Table 1).

As has been the case in many instances, the actual development of biological control of a particular pest has not been as straight forward as one might assume. Similarly I envision with regard to dwarfmistletoes certain obstacles that may make the testing and development of a biological control by either an insect or disease somewhat of a challenge. Below are listed some of the characteristics of dwarfmistletoes that may cause some difficulties in developing a biological control.

The difficulties that may arise when considering biological control of dwarfmistletoe or any pest for that matter are not presented to discourage efforts at biological control. On the contrary they are intended merely to point out objectively some of the difficulties that may arise when considering a biological control approach to a given problem.

At least two studies have been made in an attempt to achieve some measure of control of dwarfmistletoe by using pathogenic fungi as biological control agents (2, 3). These investigations were conducted in the field and were for the most part merely attempts to establish native fungus pathogens in areas where they did not naturally occur. These preliminary attempts failed to provide any measure of biological control and were not pursued any further.

Recently, however, some interest has arisen over the biological control of dwarfmistletoes. An active program aimed at the biological control of dwarfmistletoes with insects has been undertaken by Dr. Carl Huffaker and his staff, Department of Biological Control, University of California, Berkeley. These investigators are studying insects in Canada that may be introduced into California and western North America as biological control agents. To date two insects have been found that are considered possible control agents. One is a Geometrid, the other a needle miner in the genus Recurvaria. At present these insects are being studied only in their native habitat of Canada, but it is hoped in the near future that the insects may be introduced into California for testing. Plans have also been made by Huffaker to study insects of dwarfmistletoe in India as possible biological control agents of North American dwarfmistletoes. At present, however, this investigation has been temporarily postponed.

With the exception of the current program of Huffaker and staff and a few limited investigations being made on fungus parasites of

dwarfmistletoes, the biological approach to dwarfmistletoe control is not being pursued. This apparent lack of interest and activity in the biological approach to control is in contrast to the rather extensive studies and efforts being made on silvicultural and chemical control of these parasites.

I believe, that because dwarfmistletoes are the number one forest disease problem in the West and because no overall economically effective method of control has thus far been developed for them, all avenues of research leading to control should be vigorously pursued. This should include studies in biological as well as silvicultural and chemical control.

We in forestry particularly in forest pathology should at least understand that biological control is more than just a concept but an accepted, proven method of control in many instances. As researchers we should also be aware of the possible uses of biological control in our areas of investigation and disease control.

Lastly I would like to emphasize the potential use of biological control in forestry in general as a possible approach to solving many of our weed, insect, disease, and brush problem.

CHARACTERISTICS OF DWARFMISTLETOES TO BE CONSIDERED IN DEVELOPING BIOLOGICAL CONTROL

1. Dwarfmistletoes are obligate parasites and must be handled on their hosts.

2. Host specific species and forms of the parasite exist.

3. Dwarfmistletoes are widespread and grow under different environmental conditions.

TABLE 1

APPROACHES TO BIOLOGICAL CONTROL OF DWARFMISTLETOES

A. Eradicate.

   1. Kill entire plant - (resin disease).

B. Prevent spread.

   1. Kill shoots of the plant (Colletotrichum, Septogloeum, insects)

   2. Prevent flower or fruit production (Wallrothiella)

   3. Kill seed or prevent them from infecting host (fungi, insects).

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BIOLOGICAL CONTROL OF ROOT DISEASES

G. W. Wallis

The foregoing papers have given an adequate explanation of the concept of biological control. It now remains for us to discuss the principles and uses of this concept in the control of root diseases. This indeed will be a formidable task in 20 minutes, for not only must we be acquainted with root diseases in the field of plant pathology but we must also be intimate with the large volume of information disseminated in latter years in the fields of soil microbiology and fungus ecology. The approach I have chosen therefore, is to set forth certain principles, point out how they have been used and abused, and finally to summarize the future needs in research for a proper understanding of the soil and host as they relate to biological control of root disease fungi.

Fundamental to any consideration of control is a precise understanding of the growth habits and habitat requirements of the organisms involved. In no case is this truer or more difficult to acquire than in the control of root diseases.

Based on their growth habits, Garrett (1956) has divided root disease fungi into two categories:

Soil-inhabiting fungi - those characterized by the ability to survive indefinitely as soil saprophytes.

Root-inhabiting fungi - those characterized by an expanding parasitic phase on a living host plant and by a declining saprophytic phase after its death.

Representatives of both groups occur among the root parasites attacking trees. The importance of knowing into which group a fungus falls is self-evident. In the root-inhabiting fungi, which make little or no free growth through the soil, any change in the environment will probably have its effects felt through the host or host-fungus relationship. Whereas, in the soil-inhabiting fungi, environmental changes are more likely to have a direct influence on the fungi themselves.

A major proportion of the early studies in biological control of root disease fungi were conducted on agricultural crops and were directed toward soil-inhabiting fungi. A knowledge of the principles revealed by many of these studies is a requisite to a proper understanding of root disease control, particularly in nurseries.

Root disease investigations, as we now know them, go back little beyond 35 years. Earlier investigators were occupied primarily with the taxonomic identification of important root disease fungi. The work of Millard (1921) and Sanford (1926) forced plant pathologists to acknowledge the importance of the general microbial population of the soil; Millard in showing that potato scab (Streptomyces scabies) could be controlled by green manuring and Sanford by suggesting that the control might be due to the antagonistic effects of saprophytic bacteria developing on the decomposing green manure. Weindling (1932) did much to stimulate the study of microbial interactions when he demonstrated the parasitism of Rhizoctonia solani by Trichoderma.

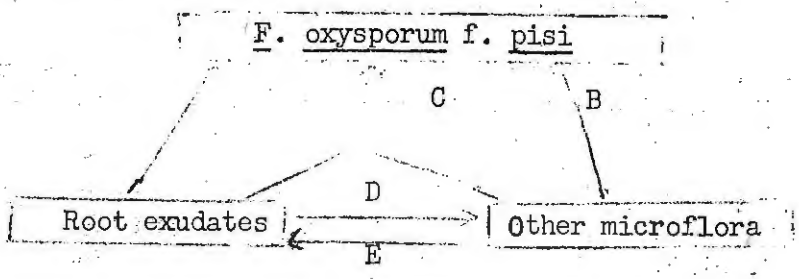
Following these early discoveries there had been extreme optimism that soil-borne diseases would soon be controlled by inoculating the soil with antibiotic producing organisms. This optimism soon faded. Garrett (1959) had this to say: "If an alien organism with such desirable characteristics be introduced into a soil population from which it is absent, there is always a chance, albeit somewhat remote, that it will succeed in establishing itself, and that beneficial results will follow. But the population of most soils is a remarkably cosmopolitan one, and the absence or scarcity of a particular organism generally denotes that conditions in that particular soil are unfavorable for it, rather than that no attempt at colonization has been made, because of its restricted geographical distribution. Most attempts at soil inoculations have had a still smaller chance of success, because the inoculant organisms had been isolated in the first place from the soil in which it was desired to establish them at a higher population level. Such attempts to boost the population of an antagonistic organism by inoculation alone have been doomed to failure from their inception, because they are in flagrant contradiction to the ecological axiom that the population is a reflection of the habitat, and that any change due to plant introduction without change in the habitat must be a transient one".

A promising approach to biological control, and one which is still being investigated today, is the alteration of soil conditions to favor the activity of saprophytic organisms, increasing the intensity of their natural biological control of root parasites. The addition of organic materials to the soil to cause a rapid increase in the numbers of saprophytes which may hinder parasitic activity is commonly advocated as a method of changing soil conditions. The early belief that the by-products of the increased populations of saprophytes arising from this procedure act directly on the parasites, however, has proven to be an oversimplification. The actions involved, are, in fact, extremely complex, encompassing the host as well as the fungi, and much has yet to be learned before the complete action is understood. Partial sterilization of the soil with chemical or steam has shown promise as a method of changing soil conditions. The organisms which first recolonize the soil following treatment govern the success of this procedure. Trichoderma viride, an active antibiotic producer when in pure culture, has been shown to have a considerable tolerance to a range of chemicals used as soil sterilants and frequently dominates the soil population following partial sterilization using steam. Although the details of all the processes involved are not yet known, the control of root parasites in nurseries by the addition of acids to alter soil conditions probably owes a portion of its success to microbial interactions.

Biological control has found limited application to date in restricting the development of root-inhabiting fungi of sapling and larger trees, however, the results of the studies involved have contributed to the overall understanding of host-fungus interactions. By ring barking trees prior to felling, Leach (1937, 1939) was able to limit the incidence of Armillaria mellea infection in the stumps and roots. This he attributed to a depletion of the starch reserves and to a rapid killing of the tissues, with the result that the roots were colonized by soil-inhabiting fungi to the exclusion of A. mellea. Bliss (1951) observed that A. mellea in citrus root sections buried in the soil was killed when fumigated with carbon disulphide. He postulated that the fungus was not killed by the direct action of the fumigant but indirectly by the antibiotic action of Trichoderma viride, the population of the latter having increased markedly in the treated soil. Although his hypothesis was shown to be only partially correct (Garrett 1957, 1958), the findings served to stimulate a great deal of research into the use of chemicals to promote rapid increases in the population of certain soil-inhabiting organisms, the development of which would inhibit plant parasites. Rishbeth (1950) suggested the the rapid spread of Fomes annosus in alkaline versus acid soil may be the result of fungal interactions. He noted that the fungus grew rapidly along the bark surface of roots in alkaline soils while in acid soils it was confined to the inner tissues. The lack of fungal growth on the bark surface of roots in acid soils he attributed to the presence of antagonistic organisms, particularly Trichoderma viride, abundant in acid environments but not in alkaline. Fomes annosus however, has been responsible for considerable damage in stands growing on acid soils in regions other than East Anglia.

Prevention of spore infection of stumps, the means by which F. annosus spreads rapidly through and between stands, may be achieved by both direct and indirect biological methods. Peniophora gigantea, when sprayed on the stump surface, will colonize the tissues to the exclusion of F. annosus. Chemicals which favor the growth of secondary wood destroyers while inhibiting F. annosus are being used and new ones are continually being sought.

An area of study, yet in its infancy, which should provide many of the answers to questions pertaining to the biological control of root diseases is that concerned with the interactions of host and organisms in the root rhizosphere. The ultimate success or failure of a parasite, prior to entering the host, is frequently determined in the rhizosphere zone. A higher population of microorganisms in the root zone as compared with the surrounding soil has been recorded on a number of occasions. Lochhead, Timonin and West (1940) and Timonin (1941) recorded differences in the rhizosphere microorganism population of wilt resistant versus susceptible flax. Buxton (1958) was able to bring about a change in the virulence of Fusarium oxysporium f. pisi by exposing the spores to the root exudates of wilt susceptible and wilt resistant peas. A good example of some of the interactions which may occur in the root rhizosphere is given by Buxton (1960). An alteration in any one of these interactions may act for or against a root parasite. "The direct effects of pea-root exudates on this fungus, (Fusarium oxysporium f. pisi), may be mitigated by the metabolic products of prevalent rhizosphere microorganisms. These, in turn, may have additional effects on the Fusarium depending on whether or not root exudates may increase their growth rates or their output of antibiotic metabolites."



"Root exudates can directly affect both the pathogenic Fusarium and the other rhizosphere microflora (A and D). The microflora will affect the Fusarium (B) and, when supplied with nutrients contained in root exudate, they may have additional effects (C). Interactions A, B, and D are primary, whereas C may be additional to B, depending on the availability and type of nutrients in the root exudate. Whether or not effect E can be assessed, that is whether any of the rhizosphere microflora may alter the quantity or quality of root exudate, depends on the evolution of a suitable technique." In his experiments, Buxton was able to demonstrate that root exudates from susceptible pea hosts which encourage the pathogen's growth or promoted germination of its spores may at the same time increase the inhibitory potential of its competitors.

These are but a few of the principles which have evolved from the extensive experimentation to date. Success in developing a satisfactory biological control procedure for many root disease fungi demands an intimate knowledge of the host and the multitude of soil organisms as well as the parasite. This would imply a much closer working liason between plant physiologists, biochemists, plant pathologists and soil microbiologists than exists at present. Practically nothing is known about the way in which root exudates, and microbial metabolic products affect microbial interactions and the host-fungus relationship. The saprophytic behaviour of most root-infecting fungi is little understood. Information on the relationship between normal rhizosphere microorganisms and parasitic fungi is fragmentary. Our basic knowledge must include a better understanding of the nutritional requirements of the organisms involved, including the synthesizing capacity of the rhizosphere organisms as well as the pathogenic fungi. Success in the future probably depends more than anything else on the development of refined techniques which will make possible the measurement and analysis of complex root exudates and microbial metabolites. Until interaction phenomenon; organism on organism, organism on host and host on organism, are known with more certainty, development of suitable biological control procedures for root disease fungi will remain in the hands of madam luck.

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PANEL II - THE ROLE OF ANTIBIOTICS IN CONTROL OF TREE DISEASES.

CONTROL OF RHABDOCLINE NEEDLE-CAST BY ANTIBIOTICS

L. C. Weir

The needle-cast of Douglas fir caused by Rhabdocline pseudotsugae, Syd. is a serious disease affecting natural and planted stands throughout North America and Europe. On this continent most concern is felt in Christmas tree production where infection renders trees unfit for sale. Earlier attempts at control were not completely successful either through a failure to affect the pathogen or for economic reasons. Work in the control of white pine blister rust, being reviewed at length at this conference, gave some hope that a measure of control might be established through the use of antibiotics.

An area in the East Kootenay region of British Columbia was chosen for the experiment and a total of 365 trees tagged for treatment. Criteria used in tree selection were: susceptibility, based on foliage loss from infection over previous years; tree height, limited to 15 feet to facilitate both adequate coverage by foliar applications and the necessary needle sampling after treatment; and relative isolation from other treatment trees to lessen the possibility of spray drift.

Two antibiotics, Phytoactin and Acti-dione (cycloheximide), plus a number of chemical derivatives of cycloheximide were applied to trees either as stem sprays or as foliar applications. All materials were applied in concentrations of 100, 200, and 400 ppm with the cycloheximide complement also applied at 800 ppm. It was felt, initially, that the highest concentrations might be phytotoxic but that it was necessary to try and cover the range within which control action might be found. All spraying was done in 1960 with a 1-gallon Dobbin hand-sprayer, with each combination of concentration and method applied to 5 trees for a total of 350 trees. In addition the stove oil carrier for the stem sprays and the stove oil-water emulsion for foliar sprays were applied alone to groups of 5 trees each and a further 5 trees were left untreated.

The assessment of any disease control experiment involving the application of fungicides to trees requires an evaluation of materials used in terms of both tree and pathogen response. With diseases such as Rhabdocline, this evaluation is best, albeit laboriously, carried out by needle counts, since phytotoxic reactions involve both foliage loss and perhaps tree mortality. In the so-called shotgun approach to control in which a range of concentrations and materials is used, both reactions may be expected with confidence.

Yearly evaluations of the experiment have been made since 1961 and during this period of time tree mortality following stem treatments

has continued to be well in excess of that after foliar sprays. The 1964 data showed that mortality rates with the respective methods of application were 60% and 11%. The majority of these deaths occurred following treatment with 6 of the 9 materials used, and rendered invalid any statistical treatment of data from needle counts.

Where needle counts were valid, the four-year accumulation of data showed a slight trend toward loss of effectiveness. Percentages of infected needles on treated trees were still below the values on the untreated trees. The data suggest that 5 years may be the limit beyond which control of the pathogen cannot be maintained, but since this time limit approaches the period of needle retention in Douglas fir, the objective of the experiment can be said to have been achieved.

Conclusions derived from all the data collected are very similar to those made after the initial evaluation. Percentages of infected needles are low enough in most cases to enable one to consider the successful materials effective in control of Rhabdocline. Of the two methods of spray application, the foliar treatments still provide the better results both with respect to tree mortality and to infection levels. It is probable, even yet, that not all the tree mortality recorded with either method results completely from treatment application although such application contributes in large measure. One is forced to rule out several materials applied either through a continued or developed lack of control of the pathogen, or through high mortality rates. Initially the compounds selected as being the most effective were cycloheximide thiosemicarbazone and Phytoactin. The data gathered over the period of examinations indicate that these two materials are still the better compounds but that the action of Acti-dione BR concentrate warrants its inclusion on the list. There remains the indication, only slightly suggested by the data that initial concentrations were in excess of the level necessary for control. Unfortunately, the nature of Rhabdocline being what it is, tests of effectiveness can only be made under field conditions and an hypothesis about lower concentrations has no basis in fact at the moment.

However, since the data also indicate that higher concentrations gave no better results than did the lower, except in isolated cases, the advocation of a fairly wide range of concentrations is unwarranted for a repetitive experiment. A further experimentation with similar materials applied to Rhabdocline could profitably be limited to lower concentrations applied as foliar sprays.

PROBLEMS IN EVALUATING RESULTS OF ANTIBIOTIC TREATMENTS FOR CONTROL OF TREE DISEASES

Charles D. Leaphart

In addressing my topic, "Problems in evaluating results of antibiotic treatments for control of tree diseases," may I suggest that our success with chemotherapeutants will depend on how thoroughly we understand three factors about the diseases we work with. These three factors are: (1) the physiology of the pathogen, (2) the physiology of the host, and (3) the host-parasite relations as disease symptoms progress through the death of the host or the peak of disease expression.

I have five one-word questions which form the basis for evaluation of antibiotic treatments. My questions are "what, how, where, when, and who." Let me explain these in order.

What. What are we evaluating in control--eradication (killing), arrestment, or prevention? All three cases might be applicable to the blister rust disease and depend on age of pine stands and management plans for these stands. For Rhabdocline pseudotsugae, it may simply be one of prevention. Regardless of the control objective, the problems have just started once your decision is made.

How. How do we evaluate control? For the culturable facultative pathogens, such as R. pseudotsugae, we can and should use isolation techniques, especially as the ultimate procedure in evaluating experimental tests; and they could very well be employed in spot sampling control programs. However, for the obligate parasites, such as the Cronartiums including C. ribicola, isolation techniques are presently unavailable, so other procedures must be used.

The presence of disease symptoms and changes in these symptoms offer other criteria for evaluation; but then do we recognize all the variations in expression of infection. For Rhabdocline, it seems that the absence of fruiting does not mean that needles have not already been infected for several years or that the mycelium could not remain vegetative following treatment. If, as with basal stem treatments for blister rust, treatments depress fruiting or if evaluation is based on only a few symptoms, interpretation of the effect of control may indeed be confounded.

For the blister rust disease, many symptoms of canker activity can be used for evaluation; but these symptoms show great variation and the absence of any activity on a canker face does not necessarily mean a canker is inactive or dead.

At least one other alternative is open to us for evaluation purposes, namely, micro- and macroscopic staining. Radiological methods are suggested as a potential means from studies of Elytroderma deformans. Our studies of blister rust mycelium concern vital staining, fluorescent microscopic, and respirational comparisons of infected and noninfected tissues. All of these studies on blister rust involve the biochemistry

and physiology of the causal organism. Although I believe we can develop at least a microscopic technique, I want to emphasize these needed fundamental studies have started many years after we started testing antibiotics. It has become clear that these studies are now needed to shorten the time period to evaluate control.

One other question concerning "how" has become very apparent in our work on blister rust. How does one separate control caused by an additive, such as antibiotics, from natural biological control? Although we often know too little about some of our forest pathogens, we know even less about their parasites. We know far less than we should about those of C. ribicola.

Ray Hirt (1) points out that many white pine blister rust cankers in eastern white pine can remain apparently dormant for several years and their symptomology is so altered that they "are difficult to recognize as blister rust cankers." In the western white pine type, we currently find a high proportion of "lethal" cankers with identical symptoms on some areas that have received no antibiotic treatment. These cankers often are infected with the purple mold fungus, Tuberculina maxima, a parasite of the blister rust fungus. In many areas nonparasitized cankers are difficult to find.

Kimmey has experiments planned or underway to assess the effect of this parasite; but, for the purposes of the present discussion, symptomology of nontreated cankers infected with Tuberculina is so similar to symptoms of treated cankers that any treated canker, parasitized with Tuberculina, cannot be properly evaluated so far as the effect of the antibiotic is concerned, unless appropriate nontreated controls are available for comparison. Some Tuberculina infected cankers, displaying inactive symptoms, go on to produce typical blister rust symptoms the next year; other infected cankers develop callus tissue.

One summary point on this subject of parasitism is that I am certain our control people care very little what is killing cankers as long as they are killed. But until the effects of Tuberculina and other biological agents and ecological factors are evaluated both with and without treatments by chemicals, including antibiotics, we will not be in a satisfactory position to evaluate effects of the chemicals, which we apply. Certainly, parasitism will be encountered in other "white pine" growing areas, and it merits consideration in your evaluation procedures.

Finally, in trying to answer the question, "How," the design for evaluating results must be statistically sound so that results can be reasonably interpreted by anyone. Your design must allow you to isolate the effect of the active ingredient, it should consider the natural variation of the pathogen and host (i.e., to incorporate as much biological knowledge of the target organism as is available), and it must weather the element of time as set by the control objective. Experiments or pilot tests that do not have adequate checks and controls or

that are violated by subsequent innovations, which were not in the original design, will rarely, if ever, be interpretable or accepted by our professional society.

Where. Where should evaluations be made on diseased hosts? Until a few years ago, we would have taken the evidence of no fruiting or nonviable mycelium in needles as possible indicators of control of E. deformans. Now we must make certain that the mycelium in twigs is also dead before control (eradication) can be claimed. Limb rust is a disease about which we also knew little concerning the distribution of its causal agent within hosts until just recently. We know now that viable mycelium may be 20 to 30 rings deep within the stem, that no mycelium exists in the several outer rings or in the phloem of stems and large branches, and that it may be found in the outer annual rings of the xylem and in the phloem only of small branches and twigs. It seems to me that this is the type of information we need before we embark on a systemic or direct application chemical control program and, more to the point of my paper, before we can do a very effective job of evaluating effects of chemical control.

As I previously mentioned, time is our only reference now in evaluating blister rust control results. Even good marginal callusing is not adequate evidence of control (eradication). Even if we had a good microscopic staining procedure of differentiating between live and dead mycelia, where and how intensively should one sample for canker activity?

It would, indeed, be very helpful to diagnose treatment effect soon after treatment on an individual canker basis, particularly in experimental tests. However, there may be good reasons to evaluate success only after a period of "x" years and just on the basis of percent of infected trees surviving after treatment as compared to percent of survivors with no treatments. Surely to be considered, for example, is the obvious fact that one surviving lethal canker, partially or totally active, on either a basally or an aerially treated tree normally spells doom to that tree whether 2 or 100 other cankers were controlled in it.

For interpretation of control of blister rust, evaluations based on either cankers or trees can produce two entirely different meanings. The degree of control in trees depends on the number of lethal cankers per tree and on the percent of cankers controlled. To illustrate, if the trees in a stand have only one lethal canker per tree, 70 percent control on a tree basis can be attained with only 70 percent canker control. But where two or more lethal cankers are present per tree and if 100 percent canker kill in 70 percent of the trees is desired, the level of canker control must range between 84 and 100 percent, depending on canker number. Thus, it seems to me that evaluation for blister rust, as well as for other diseases, should be on a tree basis; this would smooth out the natural variation in a population if the evaluation sample is large enough.

When. When should evaluations be made? If you base your evaluation on macroscopic characteristics of a disease, you must know the host-parasite relations and symptomology of your target disease. Some of the illustrations I have already used may suffice, but blister rust is again used as an example.

Hirt (2) points out and our experience confirms that in rough-barked trees, as mature trees often are, the typical blister rust symptoms of discoloration and pycnial scars are often absent. Unless cankers are examined just at the time aecia are produced, it is difficult to judge whether cankers are living, dead, or inactive. Even active cankers may sparingly, or even may not, produce aecia; so this symptom expression is of questionable value from year to year. Many of you have probably looked at more cankers than I have, but there is considerable season-to-season and year-to-year variation in discoloration, pycnia and aecia production, fruiting of Tuberculina, and various combinations of these symptoms. Thus, when we consider the question, "When," it may be determined as "x years" following the last recorded activity of a canker.

Considering this last aspect, "time," a word of caution is in order and I have briefed it previously. From our observations, a treated canker may appear inactive for several years even to the point that marginal callus has formed, but the fungus can still be active and may later fruit at various points beyond the callused margin. This reactivation (or perhaps more truly incomplete control) has been observed beyond calluses on nontreated, Tuberculina parasitized cankers as well as on cankers of trees basally treated with antibiotics. The reason for this incomplete control is not known, but that it can happen is pertinent to evaluation procedures.

Who. Who should make evaluations? To me, this aspect of evaluation is just as important as any other phase of the experimental test or control operation. If your evaluator is not a skilled, well-trained observer, does not know the variability of the disease syndrome and a superimposed disease-treatment complex, does not have close competent supervision, and is not working from a statistically, carefully planned and unbiased design, the worthiness of his evaluations will always be subject to question.

LITERATURE CITED

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TECHNICAL PROBLEMS IN CONTROL PROGRAMS

Virgil D. Moss

Technical problems in the development of antibiotic spray methods to control blister rust, Cronartium ribicola Fischer, on western white pine, Pinus monticola Dougl., as an example, have ranged from fungicide screening to spray equipment development. The sequence of steps followed to develop spray methods and the procedures to evaluate antibiotic treatment results has included: (1) fungicide screening, (2) concentrate, carrier, and additive selection, (3) active ingredient dosage and spray volume levels, (4) application methods, (5) treatment adaptation for various stand ages, (6) laboratory controls, (7) evaluation procedures, and (8) spray equipment development.

From 1949 through 1964, 76 fungicides were tested against blister rust on western white pine. Forty-seven were antibiotics and 29 were conventional fungicides. Cycloheximide and Phytoactin antibiotics were both biologically active against the rust and systemic in western white pine. Phleomycin, Murventin, Stendomycin, and Vancomycin remain to be evaluated in the nursery and field screening programs. Cycloheximide and Phytoactin formulations are available for dilution in either water or oil. Carriers were selected for compatibility, low cost, effectiveness of the antibiotic, and application facility. For basal stem application both antibiotics are diluted in Region One No. 1 fuel oil. Since cycloheximide is not compatible with all brands of No. 1 fuel oil, Triton B-1956 surfactant is added to prevent the antibiotic from crystallizing out of solution. Also, because cycloheximide has an affinity for water, spray solution containers must be kept free of moisture and spray solution applied only to dry stems. Phytoactin for foliage spray is prepared either as an aqueous solution or oil emulsion, depending upon tree age. Seedlings in the nursery are sprayed with an aqueous solution, whereas aerial spray solution is composed of 20 percent No. 1 fuel oil. Multifilm L surfactant presently is added to aerial spray solution to aid emulsification. Phytoactin aerial spray dosage and volume rates per acre are 7.5 grams and either 7 or 10 gallons depending upon stand age. Seven and 10 gallons spray solution per acre are applied to stands over 30 years and to stands 20 to 30 years old, respectively. Spray volume and stems basal treated per acre averaged 6.2 gallons and 152 trees in 1963, respectively. Phytoactin, 200 ppm, and Acti-dione BR (cycloheximide), 150 ppm, dilutions in No. 1 fuel oil are the solutions used in the basal stem treatment of western white pine. Phytoactin, 400 ppm, aqueous solution at the rate of 50 gallons per acre is applied to seed and transplant beds in the nursery. Applied in September, beds are sprayed in the evenings to take advantage of cooler temperature and higher relative humidity.

Methods of applying antibiotic solution directly to stem infections have included excise, injection, slit, and basal treatments. The basal

stem method with canker margin scarification presently is used in the treatment of western white pine. The spray procedure is to first wet stems with oil solution to aid in canker identification and outline the discoloration margin. The discoloration margin is then scarified after which generous amounts of spray solution are applied to the canker face. Trees over 10 feet tall are sprayed to a height of 6 feet. Trees less than 10 feet tall are sprayed upward to one-half their height. In addition to spraying stems, about 18 inches of the proximal ends of branches in the target zone are treated also. Aerial spray is applied with Hiller and Bell helicopters. Flight is on the contour and upon spray completion, area edges and ravines are touched up by double spray. The effective spray width is 50 feet.

The technical problem in seedling treatment is twofold: (1) to immunize western white pine nursery stock against rust infection in outplanting burned-over areas prior to or without ribes eradication, and (2) to extend protection by supplementary treatment until outplanted seedlings become of a treatable size for aerial spraying. Phytoactin applied as a foliage spray is effective in immunizing seedlings against blister rust infection. Other treatments being tested include root slurry, seedling dip, root soak, and tablet placed in planting hole. In addition to Phytoactin, the antibiotics Phleomycin, Murventin, Stendomycin, and the cycloheximide derivatives; semicarbazone, thiosemicarbazone, acetate, acetoacetate, and methylhydrazone have been tested on seedlings. Cycloheximide tests recently were terminated because the antibiotic is phytotoxic to seedlings. The treated seedlings are artificially inoculated with the rust to determine the persistence and protection period afforded by each antibiotic.

Laboratory controls are maintained on all antibiotic solutions applied in field operations. Hydrogen-ion and water hardness levels first are determined from water source samples for aerial spraying. Phytoactin aerial spray solution mixers are equipped with large capacity water softener units. Both pH and water hardness of spray solutions are periodically checked and adjusted throughout the spray season. Antibiotic absorption, translocation, and persistence in western white pine is studied by tissue extraction methods. Department of Plant Pathology, Washington State University, conducts basic research to aid in the development and improvement of spray methods for Phytoactin. Routine tests are the responsibility of the blister rust control laboratory in Spokane, Washington.

Surface symptoms on the canker face are used in evaluating antibiotic treatment results. The procedure consists of selecting only dominant or codominant crown class trees of good color and growth and with no stem canker girdling more than one-half the stem circumference. All stem cankers and all branch cankers within 4 inches of the bole are evaluated on the study trees. Canker data recorded include: stem or branch type, location, spore stage, amount of live perimeter, parasitism by Tuberculina maxima, and fresh rodent chewing. Subsequent canker growth is measured from a nail driven in the canker center to heavy type pins

placed at the four angular apex margins of discoloration. Growth is again measured and new pins added if canker margin advances in re-examining cankers over a 5- or 6-year-period.

The biological effect of antibiotics on blister rust cankers is a progressive type action evident by the symptom expression of fungus and pine. Live canker symptoms collectively used in evaluation are spore stages, bark color of canker margin, bark slippage, canker growth, purple mold fungus, and rodent chewing. The dead canker symptoms are (1) absence of all live symptoms, (2) all bark within canker area is brownish-black in color, shriveled and sunken, (3) callus tissue is visible on more than half the canker margin perimeter. Cankers that cannot be definitely classed either live or dead are placed in an inactive or controlled category. Inactive canker symptoms include (1) absence of sporulation, (2) discoloration margin is less than one-fourth the total canker perimeter, (3) there has been no advancement in mycelial growth as shown by bark discoloration measurements since spraying, and (4) rodents have not recently chewed on the canker margin.

Development of stem and foliage spray methods usually necessitates the construction or alteration of mechanical equipment to mix and apply antibiotic solutions. For stem treatment, Dobbins 145A and Hudson 291D models, 4-gallon capacity, backpack sprayers have been made adaptable for rugged field use. Bark scarifiers are constructed for attachment to the sprayer wand. Tanks with power agitation varying from 300- to 1,000-gallon capacity are used in the mixing and storage of basal stem spray solution.

Military aviation semitrailer 2,000-gallon tanks have been converted to mixers for aerial spray solution. Partitioned into two tanks, helicopters can be loaded and spray solution mixed simultaneously. Solution is mixed in the fore tank and helicopters are loaded from the rear tank. Mixer equipment includes two pumps with meters, a water softening unit, and a diactomaceous earth filter to catch silt, organic debris, and algae.

EXPERIMENTAL ANTIBIOTIC CONTROL OF BLISTER RUST ON SUGAR PINE

Clarence R. Quick

The natural range of sugar pine extends from the Sierra San Pedro Martir of Baja California in the south to Oregon in the north, i.e., to the Santiam River in the Cascades and to Curry County in the Coast Ranges of Oregon. About 80% of sugar pine cut for lumber is cut in California.

Table 1 is a somewhat generalized list of tests to date of antibiotic applications to blister rust on sugar pine. It is incomplete in that scattered and informal tests are not included. For this reason there is no totals line at the bottom of the table. The two ringed sets of tests -- involving a range of chemical, applicational, phenological, and biological variables -- probably represent the best series of small research-type tests with antibiotics that we have in California.

Test programs of the Klamath Forest and of PSW Station often have been planned as parallel and complementary series of tests. Since 1959 both series contain many tests with conventional chemical fungicides which are not included here.

First tests with cycloheximide for the control of blister rust on sugar pine were made by H. R. Offord in southern Oregon in 1952. Later small exploratory tests by H. R. Offord with rimocidin, desertomycin, and phleomycin on BR-infected sugar pine proved ineffective.

The following brief summary was received from D. P. Graham of Region 6. "(1) Some cankers on sugar pine saplings and poles have been definitely killed as a result of antibiotic treatment. (2) Permanent kill or appreciable arrestment of all lethal cankers on a large percentage of treated trees has not been demonstrated. (3) Ability of antibiotics to translocate in sufficient quantities to kill or otherwise adversely affect cankers above the treatment zone has not been clearly demonstrated in basal stem applications on sugar pine."

The following summary comments on the inter-regional test started in 1961 were received from G. M. Harvey of PNW Station. "In general, the 1964 results are no more encouraging than last year's. The percentage of uncontrolled (Class 1) cankers remains almost the same (71% this year vs. 72% last year). There has been some movement from classes 2, 3, and 4 into the Class 5 category -- apparently dead. Class 5 had 7% last year vs. 16% this year." "Inconsistency of response to treatment remains the one most 'consistent' factor in this test. On a given tree one of the antibiotics can apparently cure a canker 4 or more feet above the treatment zone. On an adjacent tree, the same antibiotic will have no visible effect whatsoever. A canker within the treatment zone and presumably thoroughly wetted with the antibiotic solution can be - - - producing vigorous aecia two years after treatment."

Table 2 outlines the codes I have used for recording estimates of tree damage and of apparent effectiveness of treatment. Regions 5 and 6 and G. M. Harvey have used the same or strictly comparable codes. The main point to note here is that the higher the numerical rating, the greater the damage to treated trees and the greater the apparent fungicidal effectiveness on blister rust infection. Mean values -- used in later tables -- are simply arithmetic averages of readings in terms of these code numbers. I have commonly included heavily damaged trees -- code 4 trees -- with the dead, code 5 trees. Average treatment effectiveness below "4.0" is considered inadequate. Average tree damage above "2.5" is considered excessive.

Table 3 summarizes 1964 data from 28 tests made with Acti-dione (1959-1962) by W. V. Showalter on the Klamath Forest. Each pair of figures -- percent of trees dead at inspection (left box), and corresponding mean treatment effectiveness (right box) -- represents a single small test. All tree mortality of 20% or more --tentatively considered excessive -- and all more-or-less satisfactory means of treatment-effectiveness (4.0 or more) are ringed. The table summarizes only the effectiveness of direct treatment. Indirect treatment -- systemic effectiveness -- has been definitely less promising.

Various inconsistencies are apparent in the table. Obviously we are not often dealing with directly comparable trees and conditions.

Table 4 summarizes data from a series of tests with cycloheximide (Acti-dione BR Conc., 5 tests, 60 trees) and phytoactin (Pabst L-341, 4 tests, 38 trees) initiated in mid-August 1960 on Hatchet Mtn., Shasta County, Calif. This area, for undetermined reasons, seems to be especially favorable for chemical treatment of blister rust.

In both sets of data note that tree mortality increased annually, sometimes discouragingly, as the tests aged. The highest treatment effectiveness attained, if consistent and predictable, might offer satisfactory control of blister rust in operations work, but tree mortality was considerable and more recent tests indicate that results are not commonly consistent and predictable.

Note especially the sizeable wax-and-wane trends in mean treatment effectiveness figures.

SUMMARY. In one word the overall summary is erraticity.

I believe in orderly cause-effect relations in natural phenomena - - - so we have apparently overlooked some major variables in our tests. Perhaps we should say that we suspect there is a joker in the log deck.

Reasons for the unexplained greater apparent effectiveness of antibiotics applied to blister rust infection on the Hatchet Mtn. area (1960) and possibly on the Mill Creek area (Klamath Forest, 1959), are largely unknown but may simply have been due to a general heavy parasitism of blister rust by Tuberculina maxima.

TABLE 1. PARTIAL LISTING OF TESTS WITH ANTIBIOTICS FOR CONTROL OF BLISTER RUST ON SUGAR PINE.

Series of Tests	Cycloheximide		Phytoactin		Controls	
	Tests	Trees	Tests	Trees	Tests	Trees
Early R5 & R5 Forests	14	128	---	---	---	---
Klamath N.F., 1959-1962	61	460	12	62	17	72
PSW-F&RES, 1959-1962	10	133	31	355	6	8
Region 5 Pilot-Type Tests	1	190	2	102	---	---
Region 6 Pilot-Type Tests	7	196	17	394	---	---
Inter-Regional Test (PNW)	24	240	24	240	8	80

TABLE 2. TREE-DAMAGE CODE AND APPARENT-EFFECTIVENESS CODE. CHEMICAL TREATMENT OF BLISTER RUST ON SUGAR PINE.

Numerical Rating	Code for Treated Tree Damage	Code for Chemical Treatment Effectiveness
5.	D - Tree is dead	E - Excellent
4.	H - Tree heavily damaged	G - Good
3.	M - Tree damage moderate	F - Fair
2.	L - Tree damage light	P - Poor
1.	N - No apparent damage	N - No apparent effect
0.	0 - Not treated	0 - Not treated
0.	θ - Can not read	θ - Not now readable

TABLE 3. 1964 RESULTS FROM 28 TESTS, ACTI-DIONE BR CONCENTRATE ON SUGAR PINE, REGION 5 FORESTS.

Conc. Acti-Dione, PPM.	Percent Treated Trees Dead at Inspection				Mean Effectiveness Rating for Direct Treatments			
	PO*	SO	SO&D	DO	PO*	SO	SO&D	DO
0	10	20	---	0	3.0	3.2	---	4.3
60	0, 0	13	---	---	3.0,3.9	5.0	---	---
100	11	---	---	---	3.0	---	---	---
120	5, 8	25	---	---	4.5,2.6	3.7	---	---
150	5, 8	---	---	---	3.7,1.0	---	---	---
200	0	9,24	20	---	---	2.6	3.5	---
300	20	15	6, 1	---	---	3.3	2.8,3.7	---
400	0	9, 5	27,18	---	3.5	4.3,4.0	4.0,3.6	---
600	---	0	25	35	---	---	1.0	4.1

*Pearl Oil (kerosene), Stoveoil, Stoveoil plus acti-dione additive "D" (=cyclohexanone), and Diesel Oil (automotive fuel).

TABLE 4. ANNUAL RESULTS FROM SOME 1960 TESTS WITH ANTIBIOTICS, BLISTER RUST ON SUGAR PINE, HATCHET MTN., SHASTA CO., CALIF.

Year of Check	Tree Damage		Sprayed Bole Infection		Unsprayed Bole Infection	
	Mean Damage	Percent Trees* Dead	Mean Effectiveness	Percent Excellent	Mean Effectiveness	Percent Excellent
ACTI-DIONE BR CONCENTRATE[1] IN STOVEOIL AND CYCLOHEXANONE[2]						
1961	2.1	22	3.4	6	2.5	4
1962	2.8	42	4.4	64	3.0	29
1963	2.8	45	4.8	75	2.9	29
1964	3.1	52	3.6	38	2.4	18
PHYTOACTIN L-341[3] IN ISOPROPANOL[4] AND OIL						
1961	1.3	8	3.0	0	2.8	11
1962	2.4	32	4.0	65	4.6	57
1963	2.5	38	3.8	38	3.8	67
1964	2.6	41	3.4	50	2.5	22

1) Five tests (200, 300, 400, 500, 600 ppm.); 60 trees.
2) Four ml. Acti-Dione Additive "D" per 100 ppm., per liter spray solution.
3) Four tests (200, 400, 400, 600 ppm.): 38 trees.
4) Only as part of furnished formulation.

ANTIBIOTIC AND CHEMICAL TESTS FOR THE CONTROL OF BLISTER RUST ON EASTERN WHITE PINE

William R. Phelps and Ray Weber

The Lake States Forest Experiment Station has established 8 separate antibiotic and chemical tests since 1962 on approximately 3,000 eastern white pine ranging in age from 8 to 25 years. Treatments were applied basally to scarified and non-scarified trunks with pressurized sprayers, by aerial applications with a helicopter, and by complete tree drenches with pressurized sprayers. Also, approximately 11,000 white pine seedlings were treated with antibiotics in complete drenches, root dips and slurries, and ground foliar sprays.

The antibiotics used most extensively were cycloheximide (actidione), its semicarbazone derivative, and phytoactin. Approximately 50 different organic chemicals and fungicides including chemical groups such as the phenolics, mercurics, dithiocarbamates, and growth regulator compounds are being tested to find an economical treatment for direct canker and scarification treatments.

Experimental Results

Basal Stem Applications - Phytoactin in three formulations (L341, 439, 440), cycloheximide, and cycloheximide semicarbazone were basally applied to 812 plantation trees that were 25 years old, averaging 35 feet tall and 6 inches DBH. Applications were made during June, August and October at several concentrations and in No. 1 or No. 2 fuel oil. Approximately 1 quart of solution was applied to the bottom 6 feet of each trunk and the first 18 inches of each branch in that area. Data were recorded from directly treated cankers, as well as those above the treated area.

After 3 growing seasons, cankers treated directly with cycloheximide in No. 2 fuel oil plus triton (300 ppm), cycloheximide in No. 2 fuel oil without triton (200 ppm), phytoactin L341 in No. 2 fuel oil (200 ppm), and phytoactin L440 in No. 2 fuel oil (200 ppm) had lengthened 3, 7, 5.2, 5.2, and 6.1 inches, respectively. Those cankers treated with fuel oil plus triton and those with no treatment had lengthened 6.9 and 8.5 inches. The majority of the cankers in the various treatments were still active.

There also were differences between treatments in canker length inhibition and canker activity scale in untreated stem cankers on treated trees. All cankers were still actively growing.

Basal Stem Scarification and Direct Canker Applications - Cycloheximide (200 ppm) and three formulations of phytoactin (L440, 414, 415) were directly sprayed or painted on scarified and non-scarified cankers in

plantation trees. These trees were 9 years old, 12 feet high, and 3 inches DBH. The materials were applied in No. 2 fuel oil, lanolin paste, and slurry paste.

After two growing seasons, cycloheximide applied directly to scarified cankers, effectively reduced canker growth and activity. There was no increase in canker length with those cankers treated with cycloheximide in lanolin and only a 0.5 inch increase in those treated with cycloheximide in fuel oil. Cankers treated with phytoactin L440 (200 ppm) in fuel oil lengthened 1.1 inches, but canker activity remained high. This was compared to an increase of 4.5 inches in untreated controls. There was apparent growth differences in the other treatments, also, but canker activity remained high.

Aerial Applications - During June, cycloheximide semicarbazone and two formulations of phytoactin (L318 and 444) were applied by helicopter to plantation trees 25 years old, 35 feet tall, and 5 inches DBH. Seven 4-acre blocks consisting of 50 test trees each were treated. The materials were applied in No. 1 fuel oil as a 20 percent oil-water emulsion at 10 gallons per acre.

After three growing seasons, no substantial differences in canker length increase were measured between treated and untreated trees. All cankers were normal and actively growing.

Complete Tree Drenches - Three formulations of phytoactin (L318, 319, 346), cycloheximide semicarbazone, and streptimidone were applied by pressurized sprayers during August as complete drenches to plantation trees 8 years old, 10 feet tall, and 2 inches DBH. The antibiotics were applied in water, or 20 percent oil-water emulsion at the average rate of 1.5 quarts of spray solution per tree.

The drenches had little or no effect in young trees after 3 growing seasons. The cankers lengthened 2 to 2.5 inches per year. All cankers were vigorous and actively growing.

Cycloheximide Bioassay - Plantation trees 25 years old, 35 feet tall, and 6 inches DBH were basally treated with cycloheximide (300 ppm) in fuel oil during June, August and October. Selected trees were sampled at intervals of increasing length, starting at one week and doubling each interval. Samples included roots, upper and lower needles, canker material, treated and untreated phloem, and treated and untreated xylem.

White pine 3-0 seedlings were root dipped or foliar sprayed with cycloheximide (50 ppm) in water. Roots, stems and needles of selected seedlings were collected from both treatments at varying lengths of time.

Samples were bioassayed for activity in the laboratory, using the standard cycloheximide bioassay procedure. Results from large trees indicated that cycloheximide was rapidly translocated, both up and down, throughout the tree although highest concentrations were in the treated outer bark and in the xylem tissues (treated and untreated). Absorption

and translocation were greatest during spring treatment and least during fall treatment. Detectable amounts were found in the trees one year after treatment. Seedling bioassays showed rapid antibiotic absorption and movement from the roots to the needles and from the needles to the roots. Detectable amounts were still found in the seedling tissues 64 days after treatment.

Summary - Antibiotic and chemical tests to date have given only preliminary results; although the trend is obvious for aerial spray and complete drench treatments. Effective control was obtained in certain cases such as with scarified cankers. This limited success promises effective chemical eradicants can be obtained through further testing of chemicals and methods of application.

THE ROLE OF ANTIBIOTICS IN CONTROL OF WHITE PINE BLISTER RUST IN THE NORTHERN REGION

Homer J. Hartman

White pine blister rust represents a billion dollar menace to the western white pine lumber economy of northern Idaho, western Montana, and eastern Washington. This rust has thoroughly demonstrated its potential to completely destroy western white, limber, and whitebark pine stands of the Northern Region regardless of stand age. Some 30 to 70 percent lethal infection is common in most stands. By the year 2000, white pine blister rust will have killed almost all unprotected limber and whitebark pine stands in this Region.

Western white pine is the most valuable and rapid growing tree species in the Inland Empire. About 2 million acres of the original 3 million acres of western white pine type remain in the current blister rust control program. Some 25 percent of these pine stands are State and privately owned.

Forty years of research, developmental work, and field observations have led to the following integrated blister rust control practices and active developments:

1. Ribes eradication on areas selected for natural and artificial regeneration.

2. Application of antifungal antibiotics to stands 10 to 100 years of age.

3. Silvicultural practices.

4. Development of immunized western white pine nursery stock through application of antibiotics.

5. Development of blister rust resistant planting stock through forest genetics.

Antibiotic testing was started in 1949. Basal stem treatment with Acti-dione BR was applied operationally in 1958. Antibiotic foliar (aerial) tests were first established in 1959 and were applied on an operational basis in 1961.

Antibiotic formulations currently being used:

1. Basal stem with canker scarification--Acti-dione BR, 150 p.p.m. in stove oil with Triton B-1956 added.

2. Aerial spraying--Phytoactin L-318, 7.5 grams per acre in 20 percent stove oil plus water emulsion with Multi-film L added is applied at the rate of 7 or 10 gallons per acre. This solution is formulated with soft water and pH is adjusted to 4.5 to 5.0.

There is no margin for error in the successful application of antibiotics for the control of white pine blister rust. For fully effective results, the required final spray solution must be correctly formulated and properly applied under exacting conditions.

The basal stem method of treatment must be conducted during the summer months and applied only when tree boles are dry. This method of treatment is generally limited to stands 10 to 30 years of age. All bole infection that can be reached from the ground must be thoroughly scarified.

Foliar application of Phytoactin L-318 is applied by helicopter during May and June and September to mid-October. The best working temperature appears to be between 40 and 68° F. with winds under 5 miles per hour, plus moderately high relative humidity. Stands treated should be between 20 and 100 years of age.

Stand acreage treated to date with antibiotics:

Basal stem	140,000
Aerial treatment	290,000
Total	430,000

Average direct per acre cost of antibiotic treatment is:

Basal stem	$30
Aerial	$11

There is a wide variation in antibiotic control results. The percentage of vigorous infections killed or inactivated by basal stem

treatment with antibiotics on an operational basis varies from 39 percent for the 1959 and prior work to 56 percent for 1961 work. Current observations indicate that 75 to 90 percent of treated infections are killed when infection margins are scarified in conjunction with basal stem applications.

Evaluations of 1962 and prior aerial spraying results show that 30 to 45 percent of the vigorous cankers have since become inactive.

Comparative information taken from untreated areas on the same basis as for hand and aerial treatments shows that 3 percent of the vigorous infections have become inactive.

Preliminary indications are that recent advancements made in antibiotic formulations and/or application techniques have resulted in an increase in infection kill.

To fully evaluate basal stem treatment results, 3 years of elapsed time are required while 5 years are required for aerial treatment.

Estimated number of antibiotic treatments required to bring various stand age classes through to commercial maturity:

Age class	No. of treatments
10-20	4
21-40	3
41-80	2
81-100	1

The exact period of white pine blister rust infection immunity that may result from various antibiotic treatments is not known. New infection has not been detected to date on western white pine over 10 years of age properly treated with antibiotics. While it is now evident that antibiotics remain active in treated pines for an unknown period, the antibiotic persistence level required to prevent new rust infection has not been determined.

No harmful side effects to fish, wildlife, or beneficial fungi have been associated with the large-scale field application of antibiotics.

In 1963 and 1964 all western white pine nursery seedlings were foliar treated with Phytoactin L-318 prior to outplanting in an attempt to immunize planting stock against rust infection for a 5- to 10-year period. At this time no specific guarantee can be made regarding immediate or long-range immunization. Current test results appear very promising.

Effective antibiotic formulations and/or application techniques still have not been fully developed for killing blister rust infections on

limber or whitebark pines. Absorption and translocation of antibiotics appear to be the main problems. Several new formulation test plots are being established each year. Presently, foliar applications appear the most promising.

The immediate regional objective is to treat with antibiotics all immature western white pine stands between 10 and 100 years of age. The problem of protecting stands under 10 years of age and over 100 years of age through antibiotics remains to be solved. The ultimate regional objective is to perform all blister rust control through aerial and hand application of antibiotics.

It is planned that through forest genetics work, blister rust resistant western white pine planting stock will be available in large quantities by 1980 and 1985.

Problems in the successful formulations and application of antibiotics closely parallel those of herbicides. There is a great potential for improvements in antibiotic formulations and/or application techniques. Much research and developmental work remains to be done.

ANTIBIOTICS ON WESTERN WHITE PINE IN PACIFIC NORTHWEST REGION, U.S.F.S.

Donald P. Graham

Magnitude

Annually, starting in 1959, we have had a modest antibiotics program. The objective of this program has been to treat selected sapling and pole stands both within and outside management units in an attempt to bring the potential western white pine crop trees through to merchantability.

A summary of the magnitude of antibiotics work on lands administered by the Forest Service and Bureau of Land Management is tabulated below. All work is included except some in special use areas and in special test plots.

Agency	Accumulative Work	
	Acres	Trees
	- - - Number - - -	
Ground application:		
Forest Service	4,158	486,095
Bureau of Land Management	395	33,648
Aerial application:		
Forest Service	3,484	401,575
	8,037	921,315

In addition to antibiotics work on Forest Service and Bureau of Land Management holdings, some application of antibiotics has been tried in Mt. Rainier National Park, Olympic National Park, and on lands administered by the Washington State Department of Natural Resources.

Results

Current results of antibiotic effectiveness on growth and vigor of selected sample cankers on treated western white pine poles are presented in tables 1 and 2. Included are evaluations from sample trees that have passed through at least two full growing seasons since treatment. Antibiotic formulations and treatment methods developed elsewhere in the West were used.

Table 1.--Current results on antibiotic effectiveness from aerial application of Phytoactin L-318 to western white pine poles.

Treatment date; acres	Antibiotic effectiveness[1)]	Basis number of cankers/ number of trees
Fall '61; 658 acres	June '63 - 30%	96/51
	July '64 - 38%	96/51
Fall '61; 104 acres	July '63 - 14%	58/29
	July '64 - 13%	56/28
Spring '62; 143 acres	June '63 - 35%	40/30
	July '64 - 28%	40/30
Control	June '63 - 3%	30/23

[1]Percent of total cankers evaluated that had definite reduced vigor with no current season sporulation and no or only a trace of active growing margin.

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Table 2.--Current results on antibiotic effectiveness from basal stem application of Acti-dione BR to western white pine poles.

Treatment date; acres	Antibiotic effectiveness[1)]	Basis number of cankers/ number of trees
Spring '59; 320 acres	June '63 - 34%	32/25
	Sept. '64 - 19%	32/25
Summer '60; 160 acres	June '63 - 7%	41/24
	July '64 - 50%	40/23
Fall '60; 73 acres	June '63 - 11%	18/15
	July '64 - 35%	18/15
Spring '61; 250 acres	June '63 - 11%	37/25
	July '64 - 35%	37/25
Control	June '63 - 12%	24/20

[1]Percent of total cankers evaluated that had definite reduced vigor with no current season sporulation and no or only a trace of active growing margin.

Remarks

1. Reaction of antibiotics on treated cankers is extremely difficult to appraise because of the inability of the examiner, by visual inspection, to accurately and consistently read and evaluate signs and symptoms of canker condition and because of the variation in appearance of a mature canker from year to year.

2. Accurate interpretation of results is further complicated because of the variation in condition of "control" cankers from year to year and because we have no positive criteria to rate each canker before treatment for comparison with canker condition at periodic intervals after treatment.

3. We have been unable to determine with consistency when decline of canker vigor is caused by a masking of disease signs and symptoms by the antibiotic and carrier, or when decline is the result of a natural or chemical healing effect on the canker.

4. To further complicate accurate readings, we find presence of the purple mold fungus, Tuberculina maxima, on from 5 percent to as high as 33 percent of our sample cankers.

5. We have no positive evidence that either of the antibiotics used in basal stem application are translocated in sufficient quantities to adversely affect cankers on the stem or branches above the treatment zone.

6. Ability of the antibiotics tried, in the manner that they have been used, to appreciably arrest canker growth, vigor, and sporulation has been demonstrated. At least this is true on most lethal cankers directly treated or most of the lethal cankers on a large percentage of the trees aerial treated. The length of time that this arrestment may last is unknown.

7. Ability of the antibiotics used--formulations and methods of application tried--to kill all lethal cankers on a large percentage of the treated trees has not been demonstrated.

8. An occasional individual canker has apparently been permanently killed as a result of antibiotic treatment. This evidence is based on complete calousing around entire canker margin with no positive evidence of canker activity outside the calloused area.

9. Although a continuing program of canker reading is needed before any final conclusions can be reached, we still do not have proof that a sufficient number of lethal cankers are being killed or canker growth arrested for a long enough period to achieve a degree of control considered necessary on western white pine saplings and poles in the Pacific Northwest Region.

SOME VIEWS ON BIOLOGICAL ASSAYS OF ANTIBIOTICS USED AGAINST FOREST PATHOGENS

S. O. Graham

The aim of this presentation is to assess some interrelationships of a set of related ideas pertaining to biological assays. The procedures used in laboratory assays on the control of plant pathogens in the field are often based on vague or easily misinterpreted criteria. Antibiotic effectiveness in the field, as measured by laboratory techniques, poses problems on what should be interpreted as valid quantitative data.

The generalizations I will make are intended as a help in the assimilation of principles, and it is hoped these opinions will meet the searching criticism of new factual information collected in the future. It is realized that modifications will be needed, although the current comments do not represent mere casual judgments. They are presented as the nearest approximation to the truth which present knowledge permits. We are pioneering in a new area to Plant Pathology, let alone to Forestry. We are in effect demonstrating the first true application of chemotherapy against fungi in perennials by the use of antibiotics.

As with any branch of the biological sciences, studies on antibiotic control of forest diseases stems from a sequence of events that developed from chance discovery of isolated and unforeseen reactions. Subsequent recognition of control commonly results from the bringing together of a group of heterogeneous and isolated cases which suggest a common underlying response. Each similar case is seen to be an example for or against the new discovery in action. The investigators who later make correlations of the data that resolve the discovery, to decide whether it is sound and usable, are able to do so usually because they employ radically new ideas, or ideas which have been formulated in an allied branch of science which can be applied to their own heterogeneous chance findings in their own field. So far, antibiotic usage against plant pathogens has been based on effective medical and clinical remedies for animals, modified to fit plant pathological practices, using medical and clinical criteria and measurement devices to evaluate the antibiotic.

This poses a basic question, "Can one equate the administration and translocation of an antibiotic in the circulatory system of animals with that in plants?" Obviously, the answer is no. Grounds for misinterpretation are apparent at once, because an immediate series of questions becomes automatic. "Is the antibiotic systemic in plants, and what do we mean by systemic?" "Can one use the same measurement criteria in different biological disciplines?" "Can one use equivalent measuring devices and expect similar results among disciplines?" "Can we correlate field applications under those attendant environments, which are normal diversities of the forest, with biological and chemical assays in the un-

natural laboratory environments?" The answers to each question are both yes and no. So long as we adhere to valid criteria to measure a phenomenon that has been created by a known vehicle, the answer is yes. As soon as criteria are modified to the point where principles are superseded or ignored as an expedient, the answer is no.

Unexpected and unexplained findings are recorded and are the constituents of discovery. However, they do not constitute discovery when these findings are forgotten. Hence, we must pursue unexpected observations to find the ultimate truth. Only when discoveries are measured do they become usable and effective knowledge. A number of explorations must be made to define parameters. From these data, correlations may then be made to prove that the original principle remains valid in distantly related applications.

We are currently in the unenviable position of exploring the efficacies of antibiotics in the control of forest pathogens, not knowing necessarily what is being measured. We do not as yet have our biological or biochemical parameters established so that necessary criteria are available in an unattestable state for interpreting the broad picture on antibiotics as control agents against obligate pathogens such as the blister rust organism.

It has been implied but not demonstrated that foliar applications of antibiotics results in a systemic distribution of a material as such. In the normal sense of the term, systemic distribution implies a translocation of the substance per se through host tissues in some discrete form. A new and unmeasured interpretation can now be made that an agent can exert a systemic effect from a distance by inducing a modification of host metabolism. Proteinaceous materials such as histones have been shown to alter the regulatory nature of the host-cell nucleolus which controls the functions of the nucleus in its coding of messenger RNA. This mechanism defines subsequent histone configurations. Such a demonstration offers a feasible means to interpret a loss in compatibility between the obligate parasite and its host. Regardless of the mode of action, the antibiotic must be demonstrated as capable of transacting the cell wall barrier as a discrete entity. This is yet to be proven for certain antibiotics.

Aside from this obstacle, there are many obstacles in laboratory assay theory which interfere with defined working parameters, let alone the erection of functional parameters for any specific antibiotic. The bioassay is of immediate concern, because we can standardize procedures and gather reams of supporting data; and yet, find we are measuring the wrong thing. There are certain inconsistencies that develop when a technique is adapted as an interdisciplinary tool and modified to fit the need.

What is the bioassay as employed, and why is it used in the way it is? It is subject to criticism and cannot be a final criterion on which to base antibiotic efficacies on rusts without reservations,

i.e., until the reaction of the parasite has been correlated to the reaction of the assay test organism. As employed it is a measure of antibiotic distribution, not necessarily a measure of antibiotic efficacy on specific pathogens.

In principle, a true antibiotic is capable of doing one of two things. It will kill or it will arrest vital activities. In other words, is a fungus spore or mycelial element dead, or is it put into a temporary state of suspended metabolism? Because a spore fails to germinate under test conditions doesn't mean that it is dead. Fungistasis vs. Fungicidal must be resolved for every antibiotic tested on every organism against which it is applied in order to fix parameters. Obviously, we use the assay as we do because we have no better tool at present. But we must not lose sight of the fact that it is a stopgap tool in our urgency to buy time by any means in our fight against insidious diseases such as rusts.

There is an elusive side aspect to be considered. Can treatments create a persistent vegetative state which defies inspection because we measure activity in terms of an environmentally induced sporulative state? We look for absence of aecia and pycnia and/or bark symptoms in Blister Rust Control as evidence of lost vital activity of the pathogen. Is the fungus really dead or merely arrested? Does the antibiotic forestall aecial production? Such a dilemma we can defer, because it dwells on the principle of practical control by reducing parasitism and pathogenesis. But where we can erect false values lies in modifying clinical measurement criteria to fit our current aims to measure efficacy of control against obligate parasites.

For example, Cronartium is an obligate parasite. It has been impossible to divorce it from its host. To breach this gap, clinical procedures have been modified and we are at the threshhold of accepting modified standard clinical criteria to measure antibiotic efficacy on the basis of assumptions. In short, we are prone to accept the philosophy that broad spectrum antibiotics induce a given specific effect.

If one concludes that an antibiotic is effective to any degree against Cronartium the amount applied is so minute that he is forced to employ an assay organism that can be grown in culture to measure the distribution pattern of the material. He must be extremely cautious at this point, however, in using it to define the efficacy of the same material against Cronartium.

It is standard procedure in any assay program to select the most hypersensitive test organism to the antibiotic that one can obtain. The assumption is that a standard curve of sensitivity to the antibiotic can be constructed for an assay organism which can be adapted to approximate the controlling dosage for the rust. On the surface this seems plausible because it works well in animal pathology. But to extract the truth, the investigator must ask several questions. "Does the antibiotic attenuate

or kill both organisms in the same way?" "Compared with lethal effects on the test organism, is it direct toxicity to the rust, or instead is it a host incompatibility relationship?" The environmental complex in the forest varies from one site to another, from one day to another, and from one season to the next. The physiologic diversities attendant to host metabolism and antibiotic efficacy against two distantly related organisms emphasizes the magnitude of error that may be introduced in interpreting field data and antibiotic application. It is easy to rationalize that an antibiotic may cause a physiologic breach such that the host-specific pathogen fails to obtain or to use the requisite metabolites furnished by the host, just as it is easy to accept a common effect of an antibiotic and ignore the fact that one is dealing with species diverse in their morphologies and physiologies, requiring equally diverse environmental conditions in order to grow. One must interpret the meaning of the nebulous term "broad spectrum antibiotic" to mean that a given antibiotic affects a large array of species, not how it affects them.

Any discrepancies in results introduced from using modified bacteriological techniques fitted to forest pathology and which are interpreted in terms of medical measurement criteria are of such great importance because they also reflect numerous variables indigenous to the assay procedure in the laboratory itself. The possible errors associated with measuring antibiotic efficacies between laboratories is gigantic unless explicit details are adhered to.

Some basic variables of utmost importance include assay conditions, the test organism, preparation of sample extractions, purity and nature of the antibiotic, and contaminating anti-metabolites in the sample to name the obvious. In the bioassay the diameter of the zone of inhibition of the test organism is the sole criterion on which concentration of the antibiotic in the sample is based. All of the mentioned variables and many more impart a direct effect on the diameter of this inhibition zone, and are known to vary between laboratories testing a given antibiotic owing to unfortunate differences in interpretation of procedures, sources of materials, etc.

As an example of conditions affecting the assay, I can point out some of them that affect the test organism with which I have been working, Colletotrichum gloeosporoides. After seeding the test organism to the plates, if the plates are chilled before application of assay discs, the zones of inhibition are smaller than if they are held at room temperature until attended. This is a variable in diffusion and creates error in assays depending on whether check plates are attended first or last after pouring and allowed to decline in temperature toward the ambient laboratory level. If the assay discs are applied to the seeded plates the same afternoon they are poured and incubated immediately, the zones of inhibition are smaller than if the plates are held at low temperatures overnight before raising the temperature to the incubation level. Another example of the diffusion variable. How long we interpret overnight is expressed in assays in which surfactants and the

type employed are used. Still another example of the diffusion variable associated with the particular solubility of a given antibiotic.

The practice in most laboratories is to prepare media ahead of time, usually a week's supply at one time or more. Media stored for two week periods have been shown to affect the growth and sporulation of C. gloeosporoides very strongly. If a prepared medium is stored under incident north light in the laboratory prior to seeding, there is a tremendous effect on the test organism and its ability to sporulate. Isolates of pedigreed C. gloeosporoides which have gone aberrant in culture have all been induced to sporulate profusely, merely by culturing them on media stored under incident north light prior to inoculation of plates. Less pronounced but similar responses can be induced by incorporating catalase or the vitamin mediator inositol in the medium. Variability in the test organism poses problems in the assay at extremely low levels. At low levels the concentration of spores is a factor and often depends on the nature of the fungus used in the assay. Many laboratories have failed to equate the number of spores in suspension when preparing seed media. If very high ratios of spores to medium are used, adsorption may become a factor, depending on the fungus employed. As an example, two test organisms are often used to assess phytoactin concentrations, Ceratocystis fagacearum and Colletotrichum gloeosporoides. The ascus of the Ceratocystis organism is coated with a pectinaceous material. Old cultures harvested for high yields of spores release large amounts of pectic materials that are incorporated in the seed medium. It has been demonstrated that phytoactin is adsorbed to pectic materials. Hence, culture age imposes a variable. With C. gloeosporoides zones of inhibition have been shown to be less if excessive numbers of spores are used in the seed medium. It has not been tested as yet, but adsorption of phytoactin to the chitinous spore wall has been suggested.

Diameter of the inhibition zone is directly affected by the type and density of the assay disc employed. The greater the ratio of cellulose to the antibiotic phytoactin, the narrower the zone of inhibition exhibited. This is a similar adsorption variable, perhaps unique to the polypeptide nature of the phytoactin molecule.

Such adsorption variables pose these same quantitative inefficiencies as a problem on extractions of samples. Furthermore, the quality and type of solvent employed varies in efficiency and ability to remove antibiotics from sample materials without denaturing the active principle. Proprietary grades of chemicals meet specified standards, but may include any other variable as an undeclared contaminant.

In the case of phytoactin extraction from pine tissues, the efficiency is increased severalfold by the manner of sample preparation. If samples are lyophilized to extract water nearly to the bound state, a great deal more activity is reclaimed than when fresh samples are dehydrated and extracted with lyophilic solvents such as methanol. Even minute amounts of free moisture present in samples appear to reduce

extraction efficiency. Obviously, humid versus arid laboratory conditions in themselves pose an extraction variable.

Antibiotics are at least initially fermentation products. The extraction of the active principle in pure chemical form from the culture milieu is extremely difficult. One of the greatest sources of potential error in working with the active principle of an antibiotic is its relative impurity as a specific chemical moiety. For example, there are six recognized penicillins, two streptomycins, two tetracyclines, and several subtilins. The occurrence of families of closely related polypeptides such as in subtilin is characteristic of polypeptide antibiotics. Hence, it could reasonably be expected to occur in one we are using, phytoactin. That it does, has been demonstrated. In what we are calling phytoactin A, the basic antibiotic molecule has a polar nature and exhibits about half the activity against the test organism as the other moiety we are calling phytoactin B. Phytoactin B is a non-polar material insofar as we can determine and is the principal material harvested from the crude extracts. Phytoactin A differs considerably from Phytoactin B in its solubility patterns and hence extraction procedures may introduce error between samples depending on the state of the tissues being extracted and the quality and type of solvents employed.

A complicating variable in host tissue extracting. Toxins and/or anti-metabolites distributed throughout the tree tissues as a consequence of mycorhizal associations has been implicated as lethal with respect to certain test organisms. Consequently, the selection of the assay organism with respect to its sensitivity to side reactions in addition to its sensitivity to the antibiotic being assayed is very important and must be defined in advance of assays.

These are some of the variables which complicate biological assays. As long as we recognize such sources of error when we interpret our attempts to measure antibiotic control of forest pathogens, our conclusions should remain sound.

PANEL III. Is Armillaria mellea a menace in forests?

H. S. Whitney - Moderator

ARMILLARIA DAMAGE APPRAISAL IN NATURAL REGENERATION OF LODGEPOLE PINE

J. A. Baranyay

Armillaria mellea (Vahl ex Fr.) Quel. damage has been widely studied throughout the world. These investigations have been mainly restricted to plantations or artificial reforestations. In these cases the hosts were handicapped by the unnatural conditions created by the process of planting, changing environment and other factors inherent in artificial culture. Armillaria damage was commonly observed in natural lodgepole pine regenerations in Alberta. In 1959 a long term project was initiated to investigate the development of the disease picture and the long term effects of the organism in natural conditions. Permanent sample plots were established in post-fire natural-regeneration in the Upper Foothills Section of the Boreal Forest Region of Alberta. The regeneration is presently 20 years old. The sample plots represent 4 different site conditions from wet to dry and a variety of stocking. Data to be discussed were obtained from surveys made in 1959, 1962 and 1964.

The incidence of root rot in living trees could not be determined accurately until 1962 because prior to then only dead trees had been uprooted and checked for A. mellea. Root rot was therefore evaluated only as a mortality factor. Mortality that was clearly resulted from Armillaria amounted to 13.7 per cent in 1959, 15.0 per cent in 1962 and 1.0 per cent in 1964. In general it seemed that tallest trees were the least affected. Conversely the plot with the shortest average tree height was most seriously affected. The higher mortality rate in the smaller trees coincided with overtopping and heavy big-game browsing. In-growth and intermediate trees were less damaged than overtopped trees. Mortality of dominants and co-dominants was very low. Stand density, therefore, seems to have been an important factor predisposing trees to serious attack by A. mellea.

2.4 per cent of the trees had been killed up to 1959 by a combination of root rot, stem rusts and non-infectious agents. Mortality of this kind had increased to 6.3 per cent by 1962 and decreased to 4.3 per cent by 1964. An indirect interaction seems to have existed between A. mellea and browsing. Seventy-five per cent of the trees killed by this combination of infectious and non-infectious agents were browsed and infected with Armillaria. All these trees were in the overtopped crown class. Browsing has contributed to overtopping and general low vigour each of which apparently predisposed trees to attack by A. mellea.

During the 1964 survey an attempt was made to determine the incidence of A. mellea on the basis of external symptoms. Discolouration of foliage, basal resinosis and reduced tree growth were measured. Fifty trees were uprooted after being examined and classified as healthy or suspect. On the basis of data obtained from this sample I concluded that chlorotic symptoms do not show up until the tree is completely girdled. A high percentage of the trees had Armillaria infection but vigorous trees localized the infections and partly or completely healed over the wounds. On the other hand, weak trees were unable to withstand the attack of Armillaria and were killed by the fungus. In most cases, basal resinosis indicated the presence of Armillaria infection on vigorous trees, whereas, poor trees did not produce this symptom. The survey showed 14.4 per cent of the living trees were Armillaria suspects. Of these 6.4 per cent were in the dominant crown class, 7.1 per cent in the co-dominant, 0.7 per cent in the intermediate and 0.2 per cent in the overtopped. This result is contradictory to the mortality data which indicated the largest damage to be in overtopped and intermediate crown classes. However, it supports the finding that vigorous trees recover. Because of the lack of resinosis in trees in the lower crown classes, this character could not be used to measure the true incidence of Armillaria in natural stands of living trees.

To investigate height growth as an indication of Armillaria infection the internodal growth of 413 trees was measured. Only 4 suspects were found in the intermediate crown class. Therefore the randomized complete block design, with 14 tree experimental units, was used to evaluate the suitability of internodal growth as an external indicator for recognizing Armillaria infected trees in the dominant and co-dominant crown classes. In the two way classification, crown classes served as blocks and healthy and suspects as treatments. A highly significant difference was found between the 10 year average internodal growth of the dominant and co-domknant crown classes. However, non-significant F-ratio for the healthy and suspects within these crown classes indicated that internodal growth was not a reliable external characteristic for recognizing Armillaria infected trees in natural lodgepole pine regeneration.

Infected living roots were cross-sectioned and the infection age determined. This ranged from 1 to 13 years. The older infections were partly or completely covered by callus tissues. Thirty root sections were used for cultural studies. Armillaria was not isolated from any of those root sections. The results of this study to the present support the view that no definite external characteristics can be associated with Armillaria root rot in natural stands of lodgepole pine.

Mortality reduced the number of lodgepole pine trees by 36 per cent on the sample plots during the 5 year observation period. Armillaria mellea was responsible for most of this reduction. While the number of lodgepole pine was reduced to 1282 on the 7, 0.05 acre plots, spruce ingrowth amounted to 1755. I did not find Armillaria

infected spruce during the survey. Lodgepole pine is a successional species in our high foothills and mountains and spruce will replace it on most of our better sites. It is suggested that Armillaria is reducing the competition of lodgepole pine, thereby giving spruce an advantage and enabling it to mix with lodgepole pine earlier, thus speeding up the succession.

SOME OBSERVATIONS ON ARMILLARIA MELLEA IN BRITISH COLUMBIA

L. C. Weir

Armillaria mellea (Vahl) Quel. is, in all probability, the most ubiquitous fungus causing root disorders in British Columbia and in other parts of the world. Observations have been carried out over a period of years on the activity of this fungus in British Columbia, although primarily on an associative basis in conjunction with other studies.

A report by Wilson (1938) referred to Armillaria in connection with dead trees on Vancouver Island but the conclusions drawn were that this root rot was probably a secondary organism and therefore not responsible for the death of the trees on which it was found.

Buckland (1953) from his examinations of young Douglas fir in both naturally and artificially regenerated stands, concluded that Armillaria attacked trees in all vigour classes. Death resulting from these attacks occurred only in trees of poor growth where bad planting practices and/or adverse environmental conditions were in effect. He also noted that heavy resinosis was associated with Armillaria attacks on trees with good vigour, but the advance of the fungus was held in check by the development of callous tissue around infection lesions. In his opinion the sealed-off lesions contained Armillaria in a dormant state capable of renewed activity whenever circumstances provided a decline in the health of the infected tree. A comparison between natural and artificial regeneration led him to believe that natural stands were much less susceptible to infection.

While some workers feel that root rot caused by Armillaria is invariably a lethal disease, these cited reports suggest a belief that the fungus is a weak parasite and actively lethal only where the effect of weakening prior to infection has predisposed the trees. Buckland infers that the factors inducing poor growth must be of sufficient duration to deny the possibility of limiting the advance of the fungus.

Records of artificially regenerated stands on Vancouver Island have been kept in the interest of forest pathology for the past decade, and

perusal of these data allow for some interesting speculation. The incidence of Armillaria, based on basal resinosis and the presence of mycelial fans, in these stands now 20 years old, has been reported to be as high as 40% in some cases. Measurements and notes on conditions have been made at intervals on a number of trees within these stands. A decline in leader height growth and increasing chlorosis was recorded for a number of years. During the past several years some of the trees have died, some have continued the decline or remained static, and others have shown an increase in leader height. This increase was construed to indicate a recovery from decline since they have, in essence, returned to a growth level normal for the stand. Comparison with weather data over the same period of time showed that climatic changes could not be entirely responsible for the decline in growth. Thus it was inferred that the decline, associated with Armillaria infection, had been overcome. Also in evidence was an indication that attack by Armillaria did not usually occur in groups of trees but rather in individual trees relatively unrelated to each other in terms of position.

It can be seen that all affected trees are not capable of isolating an attack by Armillaria. However, the apparent recovery of some is indicative that the disease is not always lethal. It must be pointed out that the existing data are limited by the availability of stands of all ages where comparative measurements might be made. Thus any hypotheses that are advanced are based on the performance within stands up to 25 years of age.

From the standpoint of a loss of crop trees, these data take on another aspect. For the purposes of illustrative conjecture, a plantation set out in 6 x 6 spacing will, at the time of planting, contain approximately 1200 trees per acre. Under forest management this plantation will receive at least one and probably several thinnings before a harvest cut is made. This is understood to be selective thinning designed to give what one might call "breathing space" to those trees destined to be crop trees. It is a further assumption, but a crop tree grown on a 100-year rotation plan should occupy, at maturity, a space roughly 17 feet in diameter. If equally spaced, these trees would number approximately 200 per acre at harvest.

These assumptions have been made on the basis of the survival of all trees initially planted, and such survival never occurs. In consequence, it follows that some of the original trees are not going to make it to the first thinning. In addition, Armillaria will account for some of those left after seedling mortality. From our data, with its mentioned limitations, there is really no reason for supposition that mortality from Armillaria will not continue for a longer period, but for the purposes of this hypothesis we will assume a slowing of effect from Armillaria beyond the age of 25. Personally, I feel that if a tree after planting survives the rigours of the new environment and its attendant problems, the odds on the avoidance of lethal Armillaria invasion are in its favour. However, provided tree loss does not occur in groups large enough to create a stand opening that

could have been occupied by a crop tree, and the bulk of our evidence is that it does not, then from the point of view of forest management, such losses are acceptable.

This conjecture is pure speculation, but it might raise interesting comments. There is not intended any inference that Armillaria is doing a job that it costs industry good money to do and that we should leave it alone.

Additional evidence of recovery in trees infected with Armillaria comes from Molnar et al (1963). This evidence was collected through observations made in the pole-blight regions of the Interior of the province and were in association with investigations of what is euphemistically called "fir decline." This decline was recorded to be affecting up to 50% of some stands. Although it was noted that some trees had succumbed, others showed evidence of the development of callous tissue around lesions, an apparent recovery from Armillaria invasion. The presence of Armillaria fans enveloping the first 6 feet of the bole of some trees that were dead was thought to have developed after death of the tree.

In conclusion, and in generalities, it is believed that, in British Columbia, Armillaria is much less likely to kill pole-size trees, but might well kill preconditioned trees up to our arbitrarily selected age of 25 years. This belief hinges on the degree of invasion, the duration of any debilitating factors contributing to tree decline in vigour, and the size of the tree involved. Two things are important for successful infection and tree death. These are the size of the attacked tree and the need for predisposition. With larger trees, preconditioning must be in effect for a longer period of time to permit complete encirclement and death of the tree than is necessary for smaller trees. Unfortunately, at the present time insufficient data are available to do more than partially substantiate this belief. However, continued collection of evidence might afford, in the future, a more elaborate hypothesis regarding the lethal properties of Armillaria.

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CONTROL OF ARMILLARIA ROOT-ROT

Lewis F. Roth

In the light of our current limited knowledge, and probably even later when our knowledge is more complete, prevention will prove a better approach to control of this disease than will cure. When thinking in terms of Armillaria control, two questions must receive consideration. First, is there a need for control, and second, have we a basis for action if control is indicated. I believe both questions are answerable in the affirmative.

I am a strong believer in the value of the natural forest as a yardstick against which the silviculturist and the pathologist can measure both their needs and accomplishments. Perhaps consideration of the natural forest is a good place to start our thinking on control of Armillaria.

The virgin forests of the world on most acres have produced prodigious quantities of wood, much of it in timber of the highest quality. But today, silviculturists aspire to exceed these yields (less is said of quality). In spite of these aspirations, it seems that for some time to come we necessarily must be satisfied with less production than the silviculturists envision, and perhaps less than the current yield of the native forest. This will be true until silviculturists can apply truly valid scientific improvements, as for example, more efficient trees. Short of this, it will be true, at least until they are able to emulate nature's process of forest production more closely; a task they often seem indifferent toward or little inclined to undertake.

One is impressed that the productive natural forest originated as an often dense stand of seedlings, in which, across the years, most of the trees of necessity succumbed, yielding their space progressively to the survivors. Very roughly speaking, we lost 50,000 trees per acre to gain 50,000 feet of prime timber. Where was Armillaria during this course of events?

We don't really know the level of participation of this fungus in stand development, and I am sure we could debate its being a constructive or destructive influence. Nevertheless, we can be sure of two things. First, Armillaria mellea was, and is, there, everywhere in the natural forest of the temperate region and probably also in the tropical forest. Second, it did not preclude development of the high yields characteristic of the virgin forest during the generation of the seedlings establishing the stands we now consider virgin. Armillaria is, and has been, in harmonious balance in the natural forest and control there is uncalled for. To be sure, there are exceptional situations where Armillaria does occur destructively in naturally established stands. For example,

in forests on immature soils given to extremes of moisture fluctuation, or areas of stress in floristic and climatic transition zones, and pockets in the natural forest following insect damage or disturbance of soil microflora or other local influence. I conclude Armillaria to be a constructive force in the natural forest, if only to provide loot for the mycophogist.

Such a statement as the preceding cannot be made of the artificially regenerated forest, or of the extensively modified natural stand. Here Armillaria is a destructive influence and control is in order if serious losses are to be avoided.

If you concur in this interpretation, can we not say, ipso facto, that it is the unnatural conditions of the artificial or extensively modified stand that are at the heart of our Armillaria problem, and that it is toward correction of these conditions that our Armillaria control efforts should be directed.

Orchardists in temperate climates and tropical planters have made some progress toward direct control through treatment of infected individuals and by rogueing the orchards and plantations. While this work may reveal principles useful to the forester, for example, Leach's work on depletion of root reserves by girdling, these expensive procedures appear inapplicable to forest practice.

As my associates on the panel have capably shown, we have a very limited base of factual knowledge of host and parasite and of their interrelationship to guide our efforts. Most of what we know concerning this disease is based on field evaluation, and often uncontrolled observation. This situation must be kept constantly in mind. Nevertheless, I believe there is enough agreement among observations of such wide origin to provide a logical if not scientific basis for action. Let us recall that Armillaria usually is widespread in the forestation site and that most woody plants are potential or actual hosts for the fungus. With these requirements met for the occurrence of disease anywhere at any time in our plantations, they can be more or less dismissed and our attention can be concentrated on the environment which appears to affect the incidence of infection and the occurrence of loss in many important ways.

1. The Environment Surrounding the Inoculum:

Armillaria is a root-inhabiting fungus whose survival and capacity for spread is influenced by the nutritional nature of the food base, by the competitive colonization of the food base by other organisms, and by moisture, temperature and other factors. These may influence survival, dormancy or active growth of the pathogen.

2. The Edaphic and Climatic Support of the Host:

First, this is a matter of suitability of the potential host

species to the growing site. In general, trees poorly suited to their sites seem the more likely to succumb to infection. One is impressed that failures away from the site of origin might be more common in species having wide geographic ranges in which there has been considerable ecotypic specialization and that species having narrower ranges fare better. It is not intended here to dismiss the planting of exotics which is not without danger, but perhaps is a special situation. A second important consideration which too often is neglected in practice, is the planting of sites that are or have become more or less unfit for planting, particularly when the planted species is the one of greatest economic importance formerly occupying the site.

3. Spatial Relationships:

The proximity of trees of the new stand to residual infected material will influence the frequency of contact with inoculum and probably the frequency of infection. Proximity of individuals will affect contagious spread. Competitive influences among the trees of the stand for use of the site will influence the liability of infected individuals to damage following infection, if not to infection itself.

4. Environment and the Year to Year Vitality of the Tree:

Year to year growing conditions affecting vitality as, for example, alternation of long periods of severe drought with long periods of adequate or excess moisture, seem especially to influence damage following infection and perhaps also the process of infection. Essentially nothing is known of the basic facts underlying this behavior. In this same category should fall many abuses of improper planting.

5. Environment and the Disease:

Environment undoubtedly influences progress of the pathogen within the infected tissues. The influence of tree age or even tissue age on infection is unknown; nevertheless, young trees once infected, are far more liable to be killed than are old trees. Conversely, if only from the basis of chance, old trees are the more liable to be infected. In older trees, root infections may be occluded by host response or infected roots may be entirely rotted off, accompanied by death of the infection. Conversely, under conditions favoring the disease, latent infections may spring into destructive activity. Death or near death of the host apparently releases the fungus for a grand period of saprophytic expansion within the individual.

Change is a distinctive feature of Armillaria expression. As the individual, the stand and the site mature, disease expression may change.

Under proper conditions, not now specifically explainable, Armillaria is aggressively pathogenic on saplings, whereas on mature timber the expression may be more one of saprophytism than parasitism.

Sudden changes disturbing the biologic balance of the stand appear especially dangerous. Figure 1 illustrates that in the presence of inoculum the trees most liable to damage are those poorly adapted to the site, or trees well adapted when growing under abnormal competitive conditions, or when weakened for any cause. Even trees of high vitality when growing competitively, may differ under a moisture regime favoring the fungus.

It would seem that our best chances for control lie in maintaining conditions just the opposite of the preceding. This essentially calls for return to a more nearly natural forest; that is, a stand with the right tree on the site, growing relatively freely throughout its life, with the minimum serious exposure to wide fluctuations of environment. How can this be accomplished?

The following considerations might be given attention:

1. Sound silviculture does not demand and should not expect on any particular site the repeated production of the tree species currently in demand by the market.

2. Clear forest harvest degrades the site and abuses of wide variety that may occur between harvest and reforestation may so damage the site as to make it unsuitable for the species harvested.

3. In the course of stand development, soils are improved by forest cover (though not necessarily for repeated production of the same species). Availability of mineral nutrients is improved, useful organic compounds are increased in supply, soil moisture and aeration are improved, and a beneficial associated fauna and flora develop.

4. Even poor sites may supply the needs of seedlings of a demanding species when the seedlings are widely spaced and not competing, but site improvement is slow during the early years and when competition sets in, the site proves deficient.

5. Probably reforestation following clear-cutting should be with a species less demanding on the site and possibly deliberately established for site improvement.

6. Spacing needs of young trees is a seriously neglected aspect of silviculture. Only natural spacing approaches the ideal (for stand health, perhaps not for growth), because it is the result of the integrated demands made by the stand adjusted on a year to year basis to the productive features of the site.

7. Natural mortality is one of the most important of the above adjustments, providing the orderly supply of new growing space to the

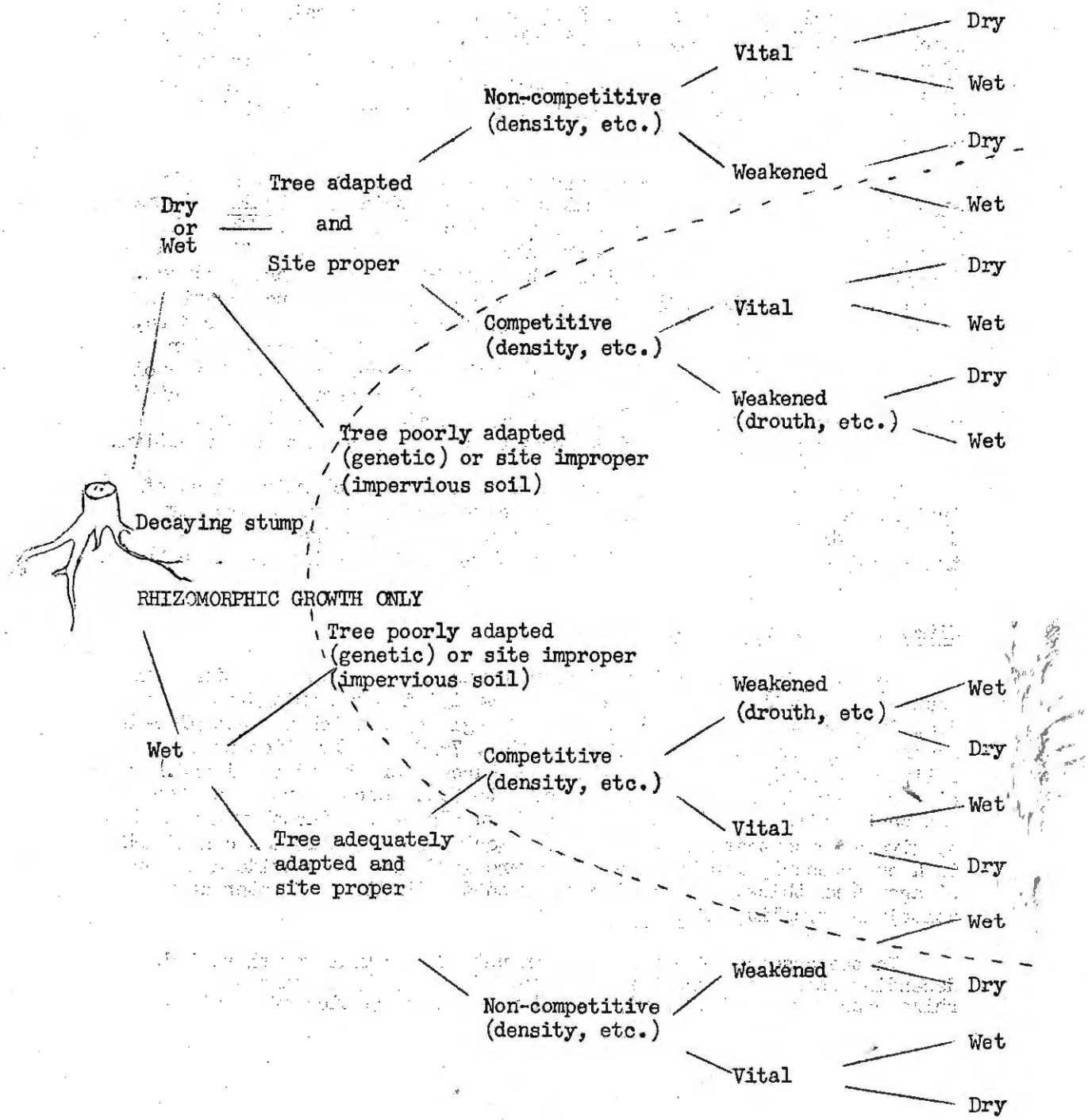
larger trees. This supply conforms to irregularities in the productive capacity of the land, and to the needs of the larger trees, not, as is so common in planting old fields and other areas free of obstacles, to some preconceived practical geometric arrangement which pays little attention to either site or silvical requirements of the tree.

As previously stated, our greatest problem appears to lie in the artificially regenerated forest. Until the scientific facts are adequately known, these procedures might advisedly be followed when dealing with the reforestation site.

1. Avoid reforestation through care and maintenance of the natural forest.
2. Where reforestation is necessary, make sure the site is right; if not right, pre-treat correctly or stay out.
3. Seed, rather than plant.
4. Reforest with a mixture of species that make varied demands on the site.
5. Selection of stock should consider species that are site-builders and species making limited demand on the site, along with species of high value.
6. If planting must be done, plant well with irregular spacing.

Clearly, for more than chance of success, the reforestation job must receive the attention of a highly competent silviculturist.

Fig. 1. Schematic representation of probable effects of environment on Armillaria root rot. The combination of conditions to the right of the dotted line especially favor damage.



NUTRITIONAL ASPECTS OF GROWTH AND RHIZOMORPH PRODUCTION BY ARMILLARIA MELLEA

A. R. Weinhold

Armillaria mellea (Vahl, ex Fr.) Quel. is an aggressive pathogen which attacks a wide range of woody plants. A unique characteristic of this fungus is its production of specialized vegetative structure called rhizomorphs. Rhizomorphs function in the penetration of host roots and are therefore very important in the pathogenic activities of the fungus.

In a recent study it was found that on a solid medium containing glucose, asparagine, essential salts, agar and thiamine, Armillaria made adequate mycelial growth but did not produce rhizomorphs. When the medium was supplemented with yeast extract or an extract of fig wood mycelial growth was stimulated and abundant rhizomorphs were produced. In addition, when the medium was supplemented with low concentrations of ethanol, extensive rhizomorph development was obtained. Therefore, ethanol would completely replace the requirement of this fungus for some factor in natural material. The optimum concentration of ethanol to give this effect was 500 ppm but a detectable response was detected when a concentration of 50 ppm was used. In addition to ethanol, 1-propanol, iso-propanol, and 1-butanol were also found to be effective.

This effect of alcohol provides a method by which rhizomorphs of Armillaria can be produced on a completely synthetic and chemically defined medium. Therefore, it was possible to investigate the nutritional requirement for optimum growth and rhizomorph production by this fungus.

Nitrogen Source Comparison

Solid medium.--The first objective was to determine the nitrogen sources most suitable for A. mellea. The initial comparison was conducted using solid medium containing 5 g glucose, which was autoclaved separately, 1 mg thiamine, 1.75 g $MgSO_4 \cdot 7H_2O$ and 20 g Difco agar in 1 liter of distilled water. Nitrogen sources were added to give 0.4 g nitrogen per liter and pH was adjusted to 5.8. Each nitrogen source was tested with and without an ethanol supplement. Ethanol was added to give a concentration of 500 ppm. Twenty ml of medium was contained in 4 oz. prescription bottles which were laid flat to provide a layer of agar 5 mm thick. The medium was seeded with discs of water agar containing mycelium of A. mellea.

In the absence of ethanol the amount of mycelial growth varied depending upon the source of nitrogen. However, in no case were rhizomorphs produced without ethanol with the exception of casein

hydrolysate after long growth periods. When alcohol was added mycelial growth was stimulated and several nitrogen sources supported good rhizomorph production. The amount of rhizomorphs was determined by measuring their total length using a planimeter.

When the various nitrogen sources were compared, on the basis of rhizomorph production, it was found that nitrate was completely unsatisfactory whereas ammonium supported good rhizomorph development. In all experiments vitamin-free casein hydrolysate was included as a standard for comparison. The nitrogen sources tested could be separated into 3 groups as follows: Very satisfactory - casein, alanine, asparagine, glutamine, aspartic acid, glutamic acid (65-100% of casein); Moderately satisfactory - serine, leucine, ammonium phosphate, and arginine (30-50% of casein); Unsatisfactory - glycine, phenyl alanine, methionine, lysine, tryptophan, valine, isoleucine, threonine, histidine, proline, hydroxyproline, and potassium nitrate (0-15% casein).

Several of the very satisfactory and moderately satisfactory nitrogen sources were compared at concentrations of 0.2, 0.4 and 0.6 g N/l. Over this range concentration of the nitrogen source had very little influence on amount of rhizomorph production.

Liquid medium.--Because impurities in agar may influence the results several nitrogen sources were compared in a liquid medium. The agar blocks of inoculum were floated on pieces of glass cloth. Under these conditions it was possible to obtain dry weight measurements of both mycelium and rhizomorphs. The performance of the compounds tested in liquid was similar to that obtained with solid medium. The initial pH of the medium did not appear to be critical over the range of 4.7 to 6.5.

The most striking effect observed with the liquid medium experiments was that in the absence of ethanol there was little or no growth of mycelium. For example, with ammonium phosphate as the nitrogen there was no growth unless ethanol was added. This observation suggested that carbon sources other than glucose should be tested.

Carbon source comparison.--In these tests a basic liquid medium containing 2 g $(NH_4)_2 HPO_4$, 1.75 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7H_2O$ and 1 mg thiamine in 1 liter of distilled water was used. Carbon sources were sterilized separately and added to give a concentration of 2.4 g carbon/liter.

It was found that ethanol was an excellent source of carbon for A. mellea. However, with glucose, sucrose, and fructose no growth occurred unless the medium was supplemented with ethanol (500 ppm). Acetate was found to be much less effective than ethanol as a medium supplement. In the presence of ethanol, glucose supported good growth while sucrose and fructose were relatively poor. An analysis of the residual medium showed that in the presence of alcohol the

sugars were utilized. In addition, growth on glucose + 500 ppm ethanol was much greater than on 500 ppm ethanol alone.

This raised the question of whether natural material would function in the same manner as ethanol. When the above medium was supplemented with either a partially purified extract of fig wood or yeast extract growth and rhizomorph production occurred. Analysis of the medium showed that the glucose had been utilized.

Summary and Conclusions.--The tests described above have shown that A. mellea will not grow in a culture medium containing glucose, ammonium phosphate, essential salts and thiamine. However, if the medium is supplemented with either natural materials or low molecular weight alcohols (ethanol, propanol, butanol) growth is abundant. A knowledge of the role of alcohol in promoting growth of A. mellea might provide some insight into the function of natural substrates.

By using a synthetic medium supplemented with ethanol it was possible to evaluate various nitrogen and carbon sources with respect to growth and rhizomorph production by A. mellea. The most extensive work was done with sources of nitrogen. With agar medium good mycelial growth but no rhizomorph development was obtained in the absence of alcohol. This suggests the presence of some impurities in the agar because in liquid medium these same nitrogen sources supported little or no mycelial growth. When the medium was supplemented with ethanol and development was used as an index of the suitability of nitrogen sources it was found that alanine, asparagine, glutamine, aspartic acid, casein hydrolysate, glutamic acid, and ammonium phosphate were quite satisfactory. Potassium nitrate and fifteen additional amino acids were either only moderately satisfactory or unsatisfactory. In liquid medium the response of the fungus to the several compounds which were tested was similar to the results on solid medium.

Only a limited number of carbon sources were evaluated. Ethanol was found to be an excellent source of carbon. When the medium was supplemented with a low concentration of ethanol glucose was a good source of carbon whereas sucrose and fructose were relatively poor. In the absence of ethanol no growth took place with any of the 3 sugars. A study of the metabolic pathways by which the 2 carbon compound ethanol, is utilized as a carbon source for growth of A. mellea is also an important area for future investigation.

SUMMATION

H. S. Whitney

The information and ideas expressed in the foregoing papers show that we are steadily increasing our knowledge about A. mellea. However, lest we begin to feel that we've about got it licked, perhaps we should ponder the following comparative statements. These quotations, paraphrases and plagiarisms have been purposely taken out of context so as to be more provocative. Apologies to their authors.

"Disease caused by A. mellea is more prevalent in plantations and in modified stands than it is in natural stands--Boyce 1961: Natural forests are rapidly being replaced by managed forests and plantations--Nordin 1961.

The fungus does not cause disease of thrifty trees--Boyce 1961: Rhizomorphs penetrate directly through sound, healthy periderm of the host--Thomas 1934.

"The basidiospores are not regarded as important in infection of the living host...."--Walker 1957: "....for though the old stump could be destroyed and its toadstools burnt, it still spread like cancer in the soil under the trees."--Large 1940.

Diseased trees occur most commonly close to old dead stumps colonized by A. mellea--Boyce 1961: When trees are close together, none escape the disease--Hartig 1873.

A. mellea invades the cambium and the tree dies--Walker 1961: Armillaria root rot weakened trees through decay and they were easily blown down by the wind--California Forest Pest Control Action Council - 1963.

Bark beetle infestations predispose trees to attack by A. mellea--Boyce 1961: A. mellea attacks predispose trees to bark beetle infestations--Peace 1962.

Induced periderm is a mechanical barrier to the further development of A. mellea, neither its toxic enzymes nor its mycelium can penetrate further--Gaumann 1950: The fungus did not become established although it had penetrated the living bark, and in some instances a secondary periderm was not formed--Thomas 1934."

It seems quite unlikely that a satisfactory understanding of disease problems caused by A. mellea will be found tucked away in some far off library. It is more apt to emerge from the efforts of energetic plant pathologists investigating the physiology of host-pathogen interactions.

PANEL IV - Adverse environment and forest diseases.

H. R. Offord - Moderator

The topic assigned to this panel is so broad in scope and impact that it might well have been called forest pathology. Adverse environment to a forest pathologist means environment that is favorable to the occurrence and intensification of a disorder, physiogenic or pathogenic.

Today we shall by-pass direct consideration of fungi in favor of two phases of physical environment and disease. In this endeavor we shall be guided by four eminent pathologists - J. A. Baranyay, G. H. Hepting, J. R. Parmeter, and Paul Miller. First, Baranyay will discuss traditional aspects of adverse physical environment by describing the problem of "red belt" a type of winter drying common in forested areas of Alberta. Then, Hepting, Parmeter, and Miller will report on the relatively new but seemingly widespread problem of damage to forests by air pollutants. Matters to be discussed here by this panel will clearly show that managers of wildlands must enlist the help of pathologists in choosing and protecting forest and park vegetation so as to minimize the impact of adverse environment.

Disease Caused by Adverse Environment in the Alberta Region

J. A. Baranyay

'Red belt' or winter drying is the most common environmental disease throughout the foothills and mountain regions of western Alberta. We had 8 'red belt' years out of the last 11, the year of 1962 and 1963 being the most severe. Slight decrease of diameter growth was indicated on the basis of increment core examination due to the loss of foliage. Growth is reduced in proportion to the fraction of foliage killed. Unusually heavy mortality was observed in two localities in 1963, where 'red belt' had occurred for consecutive years, killing merchantable timber over hundreds of acres. When the damage is lighter and the buds are not killed the reduction of old foliage predisposes trees to secondary insects and diseases which cause high mortality.

It is believed that the alternation of warm and cold air masses caused by Chinook winds are responsible for the damage. The fluctuation of temperature between -24 and 34°F for 5 days in late December, 1956 caused extensive red belting in the Kananaskis

Valley. An area was selected for detailed observation in the winter of 1963-64 because of its history of repeated red belt damage. Six hygrothermographs were deposited along the slopes with approximately 200 feet elevation differences and were run from the 25th of November to the 10th of May. Unfortunately the winter of 1963-64 was free of red belting. The largest difference between maximum and minimum temperature was 42°F for one day and the maximum temperature, did not rise above the freezing point.

A CURRENT APPRAISAL OF DAMAGE TO FORESTS FROM AIR POLLUTION

George H. Hepting

Air pollution damage to trees has been documented from parts of Germany, England, Russia, the United States, Portugal, Tasmania, New Zealand, Africa, India, South America, and reported also elsewhere. Until a few years ago damage to forests from air pollution consisted mainly of localized but very severe cases of mortality and growth loss due to oxides of sulfur or to fluoride associated with ore reduction, with a minor contribution from other sources. In recent years oxidant damage, attributed largely to ozone in Los Angeles smog, is considered partly responsible for destroying ponderosa pine in the mountains east of that city. Oxidant has also been determined as the cause of a long-known needle blight of eastern white pine now called emergence tipburn, and there is some evidence that the eastern white pine disease long known as chlorotic dwarf may be due to an abiotic air-borne agent. Mortality and growth loss to this species has also been occurring within a 20-mile radius of certain eastern power plants consuming large quantities of soft coal. New and important types of damage to trees and crops, appears to be resulting from air pollution associated with our enormous urban development, with stack gases from new industrial processes, and with greatly increased emissions of stack gases from industrial plants using fossil fuels at rates far beyond consumption only 15 years ago.

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SMOG INJURY TO PONDEROSA PINE IN SOUTHERN CALIFORNIA

J. R. Parmeter, Jr.

"X-disease" or chlorotic decline of ponderosa pine in the Lake Arrowhead-Crestline area of the San Bernardino National Forest was first noted in the early 1950's and described in 1962 (3). Detailed description of symptoms, distribution, and damage are included in the above paper.

Examination of roots, stems, and needles failed to disclose any evidence that pathogenic organisms were involved, at least in the initial stages of disease development. The random occurrence of diseased trees in residential areas, undisturbed timber stands, cut-over stands, ridge tops, valley bottoms, and lake shores suggested that drought or edaphic factors were not a direct cause. Root or stem girdling, needle removal, or fertilization with macro- and micronutrients failed to affect disease development. Reciprocal grafts between healthy and diseased trees showed that healthy scions remained symptomless on diseased trees, whereas diseased scions continued to show symptoms on healthy trees. These observations pointed to air pollution as a likely cause.

Subsequent treatment of host branches with ozone, ambient air, and filtered air indicated clearly that ozone induced typical needle symptoms whereas filtered air improved needle condition (2). These studies and additional work not yet reported implicate smog as the primary cause of X-disease. Field recordings and visual observations showed smog levels in timber stands were more than sufficient to cause the injury.

Not all trees are susceptible to present levels of ozone. Many ponderosa pines have shown no injury, even when branches are intermingled with those of dying trees. In addition, Jeffrey, Coulter, and sugar pines, white fir, incense cedar, and big-cone Douglas fir have not shown signs of injury in the X-disease area. Injury similar to X-disease and probably caused by ozone has been reported on eastern white pine in the Southeast (1).

Assuming that levels of air pollution will continue to increase with population, and this seems to be a good assumption for California, injury over greater areas is certain. Steps should be taken now to: (a) determine the relative susceptibility of coniferous species, (b) the levels of ozone necessary to cause injury, and (c) the basis of tolerance. With this information in hand, managers can know what problems to expect and how to minimize damage in future stands.

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MECHANISM OF SMOG INJURY TO CONIFERS IN CALIFORNIA

Paul R. Miller

Field Studies - Branches of two selected Ponderosa pine trees were enclosed in cone-shaped polyethylene bags. One tree was in the advanced stages of decline, the other in the early stages. One bag on each tree was supplied with either filtered air, ozone at 0.5 ppm in filtered air or ambient air. A fourth un-enclosed branch served as a control. Ozone treatment for 18 days resulted in a chlorotic mottle, terminal die-back and needle abscission on both trees similar to chlorotic decline symptoms. Ambient air treatments produced a similar result but not as severe. Branches treated with filtered air improved their green color and needle abscission ceased. Chlorophyll extractions were made from needles of different ages in each treatment and amounts were expressed as percent of control needle chlorophyll content. Chlorophyll content decreased in ozone and ambient air treatments but increased markedly in the filtered air treatments.

Laboratory Studies - Chlorophyll Decrease - Forty Ponderosa seedlings were treated with 0.25-0.35 ppm ozone 8 hours a day for 3 months in a fumigation-growth chamber. A similar number of plants were kept in an identical chamber in filtered air. Air was filtered through activated coconut carbon and ozone was measured using 2% buffered KI reagent in an impinger. The milligrams of chlorophyll per gram fresh weight was determined for each seedling spectrophotometrically in 80% acetone. The mean concentration of control seedlings was 128 mgm/gm and that of ozone treated seedlings 105 mgm/gm. This difference was significant at the 1% level.

Effect on Apparent Photosynthesis - The apparent photosynthesis of seedlings maintained in filtered air and those treated with 0.25 to 0.35 ppm ozone for 5 weeks was determined. Control and treated plants were exposed in pairs to 50 μc of $C^{14}O_2$ in filtered air for 1 hour. The radioactivity in the 80% ethanol extracts was counted. The results from two such experiments are presented in Table 1. The degree of suppression of $C^{14}O_2$ fixation was related to the decrease in chlorophyll content.

Summary - These studies have shown that decrease in chlorophyll content of needles is a symptom associated with ozone treatment both in the field and in the laboratory. Needles maintained in carbon filtered air in the field developed more chlorophyll than control needles and ceased abscission. The decrease in chlorophyll in laboratory fumigated seedlings was associated with a 38% decrease in apparent photosynthesis. The primary impact on trees showing chlorotic decline symptoms is a suppression of photosynthetic capacity which sets in motion many secondary processes resulting eventually in death.

Table 1. The apparent photosynthesis expressed as $C^{14}O_2$ fixation of seedling pairs exposed simultaneously at 83°F and 2000 f.c.

Seedling Pairs Ozone Treated/Control	CPM $C^{14}O_2$ fixed/gram fresh weight % of Control Seedling	
	Experiment 1	Experiment 2
1	30.9	49.1
2	56.4	49.9
3	63.8	59.7
4	70.5	63.2
5	74.3	64.0
6	83.7	83.3
	mean = 63.3	mean = 61.5

DISEASES OF FOREST TREES IN HAWAII

E. E. Trujillo

In the last two decades we have witnessed the most ambitious developments in the scientific fields. The accomplishments in Forest Pathology as in other areas of plant sciences have been remarkable. Disconcertingly most of the work in the field of plant pathology has dealt with the temperate zone floras while the tropical regions have received little attention. Investigations of Diseases of Tropical Forests are few and most are on forest tree species indispensable to our modern life. A good example is the work on Hevea brazilensis. We have a wealth of information on this particular species; however, if nature had endowed the ability to produce latex cheaply to some plant species of the temperate zone, our knowledge of Hevea probably would be limited to nothing else but the latin binomial. The lack of literature on disease problems of the tropical forest is somewhat embarrassing and could be attributed to limited support and manpower shortage. The situation in Hawaii has been no exception.

The state of Hawaii, because of its unusual topography, has the richest variety of climate and ecological zones in a relatively small mass of land. This makes for a diversity of forest species ranging from tropical to temperate, and from desert to alpine. In this milieu so rich and diverse diseases abound. The cooperative agreement drawn up in 1962 between the Pacific Southwest Forest and Range Experiment Station and the University of Hawaii to aid in disease identification was a much needed encouragement. This program has provided the foundation for a modest beginning in tropical forest pathology research. The accomplishments have been few, but the experiences most rewarding.

In 1963 Armillaria mellea was found killing pine sp. at elevations of 3,000 feet in the island of Hawaii. Since then this fungus has been recorded on Acacia koa, Metrosideros sp. and Grevillea robusta in the islands of Hawaii, Maui, and Kauai at elevations above 3,000 feet. The distribution of the fungus apparently is restricted to areas with cool soil temperatures.

The nursery problems in Hawaii are of great interest to us because many of our forest soils appear to be free of pathogens such as P. cinnamomi, thus disease seedlings are the best means of dissemination of these organisms throughout the forest soils. P. cinnamomi has been isolated from pine and Araucaria seedlings from private nurseries. Other pathogens such as Pythium sp. and Rhizoctonia solani are commonly found in most nurseries.

A very interesting mycorrhizal association between an ectotrophic mycorrhizal fungus and slash pine has been noticed in the state nursery

at Hawaii. When the nursery soil is fumigated for the first time, the pine seedlings fail to develop mycorrhizae and grow very poorly. This problem is being studied in conjunction with soil fumigation tests with different materials to control damping off organisms.

Botrytis cinerea is a very destructive saprophyte under Hawaiian conditions. In the nursery it is particularly serious on Eucalyptus deglupta when the seedlings are topped during cool, moist weather. Sanitation and protective fungicide sprays before and after topping would be tried to control the problem. Other problems under investigation are the dieback of tropical ash and silk oak; however, little progress has been made on these diseases. Five Korthalsella spp. of mistletoe have been described in Hawaii, but the importance of this parasite remains to be determined. Wood rotting fungi have been found on Acacia koa and Metrosideros sp. Ganoderma sp. and Polyporus sulfureous appear to be the important basidiomycetes in the wood rots.

Araucaria excelsa is used in Hawaii for Christmas tree production. However, a very undesirable bronzing of the needles has troubled the Christmas tree industry for some time. Investigation in cooperation with the State Division of Forestry in Maui indicates that this problem is physiological in nature. The cause is attributed to higher light or UV intensities occurring in the tropics. Seedlings become bronze more readily at high elevations than at sea level; also, the underside of the needles is always green while the surface exposed to sunlight acquires the characteristic bronze coloration. Experiments have shown that seedlings grown in the shade remain green while seedlings exposed to full sunlight become bronze.

The damage caused by Uromyces koa on Acacia koa seedling regeneration deserves our attention. The same can be said for the different canker type symptoms found on koa, monkey pod, and other tropical hardwoods. We expect that as funds and manpower become available more interest and time will be devoted to the solution of these problems.

SOME FOLIAGE DISEASES OF CHRISTMAS TREES IN WESTERN WASHINGTON

Jack D. Rogers

INTRODUCTION:

The production of Christmas trees in the Pacific Northwest is big business. For example, in 1959 over 3.5 million trees were cut, 85% of which were Douglas-fir (2). Although many of these trees were grown on "wild" forest lands in conjunction with other forestry activities,

a significant number were grown in plantations under intensive cultivation.

Christmas tree growers must produce a top-quality tree in order to get top prices. Foliage discolorations, resulting from either biotic and/or abiotic causes, markedly reduce the dollar value of Christmas trees. Many of these degrading foliage diseases have been ignored by timber-oriented foresters and forest pathologists. In fact, some foliage discolorations are definable as diseases mainly because they impair the usefulness of the plant as a Christmas tree. This paper summarizes preliminary research on some fungi associated with foliage discolorations of Christmas trees in western Washington.

METHODS:

Discolored foliage was collected in the field by the writer or was sent to him by collaborators. Freehand sections of discolored foliage were examined; isolations were made. Cultures were initiated and maintained on the following media: 2% potato dextrose plus yeast agar (5 g yeast extract per liter of Difco media), hereinafter designated PDYA; 2% malt agar, hereinafter designated MA; 2% malt-yeast agar (Difco), hereinafter designated MYA. Cultures were incubated at room temperature in light and dark.

An attempt was made to correlate symptoms and signs on foliage with particular organisms. This could not be accomplished with certainty because the manifestations of several of the organisms are indistinguishable macroscopically. What is more, several organisms often were growing intermingled or, at least, in close proximity. Therefore, the short descriptions of foliage discolorations are meant merely as rough guides to the general types encountered.

DESCRIPTIVE PORTION:

1. Aureobasidium pullulans (deBary) Arnaud
   Syn. Pullularia pullulans (deBary) Berkhout
Host: Foliage of Pseudotsuga menziesii.
Locality: Thurston Co., Wash.

Symptoms and signs: Forming discrete black spots on green foliage. Commonly associated with aphid and other insect wounds. Covering dead needles and needle tips with diffuse blackish growth.

Cultural and morphological characteristics: Cultures on PDYA at first brownish in center with whitish advancing zone, soon becoming blackish-brown toward center, slimy, covering plate in about 3 weeks. Reverse black. Hyphae exceedingly variable in diameter, hyaline to dark brown, some of them thick-walled and closely septate. Conidia hyaline to subhyaline, exceedingly variable in shape and size, produced from conspicuous pores in thick cell walls or budded from thin-walled hyphae, budding to form secondary spores. Large dark-colored spores ("chlamydospores"), 0-3+septate, abundant, germinating by producing conidia or giving rise to hyphae.

Remarks: The ecology of this ubiquitous fungus has been reviewed by Cooke (1). There are numerous reports of A. pullulans behaving as a secondary invader of injured foliage. Molnar (4) and others have described build-ups of the fungus following insect attacks. Ouellette (5) has reported the occurrence of the fungus on frost-injured foliage.

In western Washington A. pullulans appears to invade aphids and mite wounds. It apparently is not a primary parasite.

2. Sporotrichum sp.
Host: Foliage of Pseudotsuga menziesii.
Locality: Thurston Co., Wash.

Symptoms and signs: Uncertain. Isolated repeatedly from aphid punctures, usually in association with Aureobasidium pullulans.

Cultural and morphological characteristics: Cultures on PDYA white to yellowish, closely appressed, slimy, covering plate in several days. Conidia produced from sides and apices of hyphae, either as buds or, usually, from minute spicules. Conidia hyaline, elongate or, occasionally, tear-drop-shaped, pointed at one end, (2)5-10(13) X 1-4μ, often producing blastospores by budding.

Remarks: The role of this fungus in discoloring foliage is uncertain. It has been isolated from feeding wounds and from the exterior and interior of aphids. It is probable that the fungus is introduced into foliage by feeding insects.

3. ???Phoma sp.
Host: Foliage of Abies grandis.
Locality: Thurston Co., Wash.

Symptoms and signs: Tips of needles dead, with a sharp demarcation between brown and green portions. Pycnidia embedded, but partially and conspicuously erumpent, from undersurface of browned portion of needle.

Cultural and morphological characteristics: Cultures on PDYA, MA, or MYA growing rather slowly (about 1 cm per week), appressed, furrowed, pinkish-tan, velvety. Reverse brownish-orange. Pycnidia 100-300μ in diameter, yellowish at first, later dark brown, short-necked. Conidia broadly ovoid to globose, hyaline to subhyaline, 1.6-7(10) X 1.6-5(8). Conidia from pycnidia on needles are identical but average significantly larger, 6-10(12) X 4-8μ.

Remarks: This fungus apparently is a primary parasite. The writer is not entirely convinced that it is a Phoma, but has been unable to fit it into any other genus.

4. Cladosporium herbarum (Persoon) Link
Hosts: Foliage of Pseudotsuga menziesii and Abies grandis.

Localities: Thurston and Mason Cos., Wash.

Symptoms and signs: Forming large stromata within brown portions of needles. Conidiophores usually growing from stromal surface. Sometimes forming a diffuse brownish or greenish growth on green or dead needles.

Cultural and morphological characteristics: Cultures on PDYA greenish, with gray aerial mycelium near colony center, appressed, matted. Growth slow (4-5 cm in 3 weeks). Conidia on swellings of sparingly branched, brownish conidiophores, at first terminal (usually), becoming lateral by extension of conidiophore tips. Conidia 1-2, sometimes 3-4, celled, with minutely roughened walls, yellowish to brownish, variable in shape, but usually lemon-shaped, (5)10-16(20) X 6-8μ.

Remarks: It is suspected that C. herbarum is a weak parasite on frost-injured needles. Inoculum probably is always present in stands because the fungus sporulates profusely on decaying needles.

5. Botrytis cinerea Persoon
Host: Foliage of Pseudotsuga menziesii.
Localities: Thurston and Pierce Cos., Wash.

Symptoms and signs: Sporulating profusely on watersoaked needles just emerging from buds. Isolated from opening buds and from frost-injured foliage.

Cultural and morphological characteristics: Cultures on PDYA gray turning gray-green to gray-brown as sporulation begins, fluffy with aerial mycelium. Conidiophores unbranched or sparsely branched, up to several mm long, thick-walled, brown, bearing heads and lateral clusters of conidia. Conidia produced from tiny sterigmata, hyaline to subhyaline, apiculate at base, ovate to nearly globose, 5-13 X 6-8μ. Sclerotia produced in abundance in old (several months) cultures.

Remarks: Botrytis cinerea as a pathogen of various conifers is cited by Peace (6) and many others. The fungus is most damaging in plantations of Douglas-fir located in fog bottoms and frost pockets. It seems likely that the fungus colonizes frost-damaged foliage and, under moist conditions, attacks adjacent healthy succulent foliage. In low areas, in wet years, B. cinerea causes considerable killing of emerging foliage in western Washington.

6. Epicoccum nigrum Link
Host: Foliage of Pseudotsuga menziesii.
Localities: Mason, Pierce, and Thurston Cos., Wash.

Symptoms and signs: Small dark spots (sporodochia) or diffuse brown growth on green or dead foliage. Often associated with aphid punctures.

Cultural and morphological characteristics: Cultures on PDYA yellowish

to rusty reddish, becoming brown, with abundant fluffy aerial mycelium. Yellowish to reddish pigment diffusing into medium often well ahead of growing frontier. Sporodochia scattered on surface of agar, producing conidia from very short or obscure conidiophores. Conidia also produced on unaggregated hyphae. Conidia (15)20-25(30)μ in diameter, yellow to brown, coarsely reticulate and warty on surface, usually divided into several irregular-shaped cells by prominent septa.

Remarks: This fungus is often associated with aphids, particularly the wooly aphid, Chermes cooleyi. In several instances living aphids have been observed with spores of E. nigrum attached to their backs. It seems likely that the fungus can colonize fresh aphid puncture wounds. The fungus is widespread as a saprophyte on fallen decaying needles, thus probably assuring a ready supply of inoculum.

7. Phomopsis sp.
Host: Needles and leaders of Pinus nigra.
Locality: King Co., Wash.

Symptoms and signs: Pycnidia erumpent from bark of leaders and protruding from slits in needle epidermis. Needles reddened and brittle.

Morphological characteristics: Not cultured. Pycnidia short-necked, up to 300μ in diameter. Alpha conidia pointed at one end, rounded at the other, 1-3 X 5-8μ. Beta conidia J-shaped to crescent-shaped to almost straight, 15-25 X 0.25-1μ.

Remarks: Phomopsis sp. apparently is a primary pathogen. The plantation where it was found was overgrown with weeds, apparently creating a favorable moist environment for the fungus. Pinus nigra is not grown to much extent in Washington. It is not known if this Phomopsis is specific for P. nigra or if it is a species ordinarily found on some other host(s). More fungal material and experiments would be necessary to determine if the fungus has been described (see Hahn, 3).

DISCUSSION:

The pathogenic abilities of none of the fungi described have been proven. Biological studies, particularly inoculation studies, are needed. Collectively, these fungi are associated with a great deal of foliage discoloration of Christmas trees. Thus, pathogenic or not, they must be considered as economically important to a growing industry.

The relationships between insects and certain of these fungi- Aureobasidium pullulans, Sporotrichum sp., and Epicoccum nigrum- are not clear. It seems probable that they can colonize insect feeding wounds. In addition, it is known that aphids transport spores of E. nigrum and reasonably certain that Sporotrichum can be borne within the insect. Aureobasidium pullulans might well be carried by insects, externally and/or internally. Steinhaus (7) has reviewed some of the relationships between insects and fungi.

It is apparent that a significant amount of foliage discoloration of Christmas trees is owing primarily to insects. Growers assume that, once the insect has been removed from a plant, there will be no further problem. They, therefore, spray for insects, particularly aphids, whenever it is convenient. Often, the aphids have fed for weeks prior to control efforts. Thus, foliage often is riddled with feeding wounds, sometimes already discolored by other organisms, by the time sprays are applied. The grower becomes worried about these needle discolorations and sprays indiscriminately in hopes that they will miraculously disappear. In the meantime, insects in other parts of his holdings are ignored. Prompt aphid control will significantly reduce foliage discoloration of Christmas trees in western Washington. It is to be stressed that A. pullulans, Sporotrichum sp., C. herbarum, and E. nigrum are cosmopolitan: controls must be aimed at minimizing portals of entry, not at the fungi directly.

Damage from Botrytis cinerea, another cosmopolitan "parasite of opportunity," can be minimized by avoiding frost pockets and fog bottoms, sites which also favor the very damaging Rhabdocline pseudotsugae.

It remains to be seen if the Phoma (?) is widespread in its occurrence and damage. If so, its disease cycle should be elucidated in order that effective control measures be developed.

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TRANSLOCATION RELATIONSHIPS BETWEEN MISTLETOES AND THEIR HOSTS

O. A. Leonard and R. J. Hull

This is a summary of 4 years work on "Translocation Relationships Between Mistletoes and Their Hosts." These studies were on 8 dwarf mistletoe infected hosts and on 8 green mistletoe infected hosts. One of the hosts (white fir, Abies concolor) was infected with both Arceuthobium campylopodum and Phoradendron bolleanum var. pauciflorum. All of the tests were conducted in natural habitats.

The dwarf mistletoes and hosts were as follows: Arceuthobium americanum on Pinus murrayana; A. campylopodum on P. sabiniana, A. campylopodum on P. lambertiana; A. campylopodum on P. ponderosa, A. campylopodum on P. monophylla; A. campylopodum on P. jeffreyi; A. campylopodum on Abies concolor; and A. campylopodum on A. magnifica.

The green mistletoes and hosts were as follows: Phoradendron flavescens var. villosum on Quercus wislizenii; P. flavescens var. villosum on Q. kelloggii; P. flavescens var. villosum on Q. douglasii; P. flavescens var. macrophyllum on Juglans hindsii; P. bolleanum var. densum on Cupressus macnabiana; P. bolleanum var. pauciflorum on Abies concolor; P. juniperinum var. libocedri on Librocedrus decurrens; P. juniperinum var. ligatum on Juniperus occidentalis. Uninfected hosts were also studied for comparative purposes.

The labeled compounds used in these studies were as follows: $C^{14}O_2$ (out of which labeled photosynthate was formed after exposure to leaves in sunshine); several C^{14}-labeled herbicides (2,4-D, 2,4,5-T, amitrole, atrazine, paraquat, maleic hydrazide); C^{14}-labeled urea; $P^{32}O_4$, and $S^{35}O_4$. Label was applied to host foliage, host bark, cuts in the stems, and mistletoe shoots. Results were obtained by radioautography, counting, and chromatography. About 300 tests were conducted.

When the host foliage was treated with $C^{14}O_2$, labeled photosynthate migrated downward, with an appreciable accumulation of label in the endophytic system and aerial shoots of the dwarf mistletoes. This picture held for all of the infected hosts and for all seasons of the year. There was no evidence of any blockage of translocation by the endophytic system except occasionally during the summer period; the blockage or interference in transport appeared to be due to the food requirements of the growing host shoots and the dwarf mistletoes, leaving no extra food material to be moved to the base of the stems. This same interference occurred in one instance when the dwarf mistletoe infection occurred on a defoliated side branch; much of the label was directed into this branch which resulted in the basal portion of the main stem being unlabeled.

Seasonally, translocation in dwarf mistletoe infected hosts was most rapid during the winter and spring period before shoot growth had commenced and slowest after the shoots had started to grow, remaining slow during the summer and fall.

The dwarf mistletoe shoots did not translocate any photosynthate into its own endophytic system or into the host at any time of the year or by any manipulation of the hosts to raise its food requirements (such as by defoliation).

Amitrole applied to the leaves of Abies concolor migrated into the dwarf mistletoe infections to the greatest extent when the leaves were covered with a polyethylene bag to create a condition of high humidity. $P^{32}O_4$ was absorbed and translocated to the greatest extent when the leaves (Pinus sabiniana) were first severely scraped with a razor blade. 2,4-D, 2,4,5-T, atrazine migrated out of Abies concolor leaves only slightly.

Herbicides were absorbed but poorly through the bark; however, an ester of 2,4,5-T applied in diesel oil was absorbed in 9 months, although no absorption appeared to have occurred in 2 weeks.

By far the most effective method of applying all materials (except $C^{14}O_2$) was through cuts in the stems. The most fruitful studies were conducted with $P^{32}O_4$ and $S^{35}O_4$. The translocation following such treatment was upward, with downward movement generally being interrupted by a girdle; however, in some cases, a girdle did not prevent movement through it. The dwarf mistletoe endophytic system and aerial shoots were well labeled by this procedure; however, there was appreciable variation in the labeling of different parts of an infection.

Labeled herbicides applied to dwarf mistletoe shoots did not enter the endophytic system. Further, $P^{32}O_4$ applied to dwarf mistletoe shoots did not generally enter the endophytic system or the host tissues. However, in several instances, pricking the shoots with a pin immediately before application of the label did result in the label entering the host. In 2 cases, the base of the mistletoe shoots were first steam-killed before adding the $P^{32}O_4$; this did not prevent the label from entering the host. Evidently, in the few cases when label did migrate from the aerial shoots into the hosts, it occurred in the non-living phase or apoplast.

Phoradendron infected hosts translocated labeled photosynthate in much the same way as did the Arceuthobium infected hosts, except little if any label entered the mistletoes. A similar result was experienced when $P^{32}O_4$ was applied to the host foliage, although a limited amount of label did find its way into the mistletoes.

When $P^{32}O_4$ was applied to cuts in the stems of green mistletoe infected hosts, the mistletoe shoots became highly labeled. This was the most effective method of introducing label into the green mistletoes.

When $C^{14}O_2$ was applied to the shoots of the green mistletoe, labeled photosynthate migrated downward into its endophytic system, but only traces of label entered the host stems. The application of $P^{32}O_4$

to green mistletoe shoots gave results that were similar to that of photosynthate. In a few cases, some $P^{32}O_4$ did migrate from the mistletoe into the hosts, but these were exceptions.

Host-leaf free green mistletoe infected branches were found to obtain their nutrition entirely from other host branches. When other branches were fed $C^{14}O_2$, labeled photosynthate entered such branches in high concentration but only low levels entered the green mistletoes. When the green mistletoe shoots on such branches were fed $C^{14}O_2$, the photosynthate migrated into the endophytic system with only traces of label entering the host. Similar results were obtained with $P^{32}O_4$, except the results were not as clear-cut.

Photosynthesis and respiration experiments were conducted on the shoots of both dwarf and green mistletoes. It was found that the dwarf mistletoe does not manufacture sufficient photosynthate to take care of its own respiration requirements. However, the green mistletoe takes care of its own respiration requirements at a light intensity of 800 foot candles and manufactures food in excess of respiration requirements when the light intensity is greater than this.

SAPWOOD STAINING FUNGI IN THE GENERA CERATOCYSTIS AND EUROPHIUM

Ross W. Davidson and Robena C. Jeffrey

This project, which is supported by the National Science Foundation, was started in the spring of 1962 and will continue to June 1966.

The principal objectives of the project are to gain information on the distribution and prevalence of blue stain fungi and obtain cultures for detailed laboratory study. In the summer of 1962, collecting was conducted in the northwest United States; Idaho, Washington, Oregon, and California. In 1963 collecting was in the eastern United States; Georgia, North Carolina, Maryland, Virginia, New York, and New Hampshire. In the past summer, collecting was concentrated in New Mexico and Arizona. Travel is by automobile and small house trailer and extends over a period of about two months during each summer, from about June 15 to August 15.

These collecting excursions by the senior author have been supplemented by more intensive collecting in the vicinity of Calgary, Canada from lodgepole pine by the junior author and by collecting in Colorado before June 15 and after August 15 during each summer period.

Laboratory studies are made of the cultures during the fall and winter of each year.

It will be remembered that all of the species of Ceratocystis, or Ceratostomataceae, are disseminated by insects. Therefore, collecting has been in areas where bark beetles are most prevalent such as logging areas, and from trees recently killed by insect attack or other causes. Specimens must be collected within one year to 18 months after logging or after initial invasion by insects for most profitable isolation results. Also, collecting should be conducted before trees or logs have become thoroughly dried out. Isolation should be made from specimens immediately, before they have dried out.

Isolations are made from bark insects, from fungus fruiting bodies in the insect galleries of the bark or sapwood, or from stained sapwood.

RESULTS OF STUDIES WITH CONIFERS

In species of pine both in the east and in the west, Ceratocystis ips (Rumb.) C. Moreau is closely associated with Dendroctonus and Ips spp. bark beetles. Perhaps this fungus is most typically associated with Ips spp. beetles, but in the west especially, C. montia (Rumb.) Hunt which has usually been isolated from Dendroctonus, seems little different from C. ips. Another species almost constantly associated with Ips spp. in pines is the European species C. minuta (Siem.) Hunt. It is usually present in great abundance along with C. ips. There are several forms of C. minuta which may be described as distinct species, such as one with white perithecial base and black neck and an all-white perithecial form. All have the characteristic filiform ascospores which are forcibly ejected in long hair-like or spider web-like filaments.

Frequently a large perithecial Ceratocystis has been collected from Dendroctonus galleries in pines, especially from western white pines attacked by D. monticolae and occasionally from lodgepole and ponderosa pines in Canada, Colorado, and Arizona. One culture was also obtained from eastern white pine in N.Y. state. This is the species recently described as C. huntii Robinson in the Can. J. Botany in June, 1964. This species has a Leptographium or Verticicladiella imperfect stage. It is not known whether it is actually carried by Dendroctonus or by some closely associated secondary insect.

In many instances of isolations from bark beetles (Ips and Dendroctonus) from pines, mixtures of fungi have resulted but both C. ips and C. minuta have frequently been obtained from such mixtures.

Isolations from stained sapwood of trees attacked by Dendroctonus have often contained species of Leptographium of the sub group Verticicladiella. This is true even where C. ips-montia have been isolated from the beetles. Such wood is usually stained a very dark color. Such cultures have in the past not been identifiable to species. However, some progress has been made with this group since Parker (1957) described a fungus with a "perithecium" which he placed in a new genus Europhium. The present authors have three apparently new species of

Europhium which may be separated by characteristics of the imperfect stage. These three, along with E. trinacriforme, C. huntii, and C. europhioides Wright and Cain (1961) give us a start towards a better knowledge of this interesting but difficult group of staining fungi. We continue to isolate strains or species of Verticicladiella which cannot be definitely identified to species (some work has been done by Kendrick on these imperfect forms (Kendrick, 1962)). Ceratocystis europhioides mentioned above has been obtained and identified during this study from Douglas fir.

Ceratocystis minor Hedgc. (C. pini) has been isolated frequently from conifers (pines, spruces, and firs). It was earlier reported as associated with Dendroctonus in pines in the eastern U.S. and from Dendroctonus in Douglas fir in the west. During this study it has been observed in association with D. pseudotsugae (Idaho), D. brevicomis (California) and D. jeffreyi (California). However, it is so frequently observed in Douglas fir, spruce, true firs, and conifers in general, unassociated with Dendroctonus that its true carriers are uncertain. Perhaps there are distinct strains of this species which are consistently associated with certain specific insects but at present we have insufficient information to make any separation on this basis.

More intensive studies of subalpine fir in Colorado have shown that the fungi described by Davidson in 1958 (C. penicillata (Grosm.) C. Moreau, C. nigra Davidson, and C. brunnea Davidson) are common in this tree species infested by bark beetles (Dryocoetes and others) both in Colorado and in northern New Mexico, and Arizona. However, it is now known that C. penicillata from this host was not correctly identified. It is currently considered to be an undescribed species and has been collected commonly in Engelmann spruce as well as in subalpine fir in the three states mentioned above. Superficially, the species is similar in perithecial characteristics to C. huntii in pines but ascospores and conidial stages differ considerably.

Quite a few less common species of Ceratocystis from conifers have been isolated and studied. Some of these are C. coerulescens (Münch) Bakshi, C. pilifera (Fries) C. Moreau, C. olivacea (Mathiesen) Hunt, and C. picea (Münch) Bakshi. A number of apparently undescribed species have also been studied and descriptions of these are being developed.

RESULTS OF STUDIES WITH HARDWOODS

The hardwoods have not been studied nearly so thoroughly as have conifers but many of the common species are already known. In general, insect carriers of the hardwood species of Ceratocystis are not known.

One culture from an oak log in Mississippi has been described as a new species C. megalobrunnea Davidson & Toole. Another, from Nectria-like cankers on aspen in Colorado, New Mexico, and Arizona has been described under the name of C. tremulo-aurea Davidson & Hinds. These two should appear in Mycologia late this year.

C. fimbriata Ell. & Halst. has also been isolated from cankers on aspen by Tommy Hinds and during the present study as well. It does not fruit commonly on aspen cankers and in fact perithecia of it were observed only once during these studies.

I am familiar with hardwood inhabiting species of Ceratocystis from studies at lumber mills in years past. The common species are C. moniliformis (Hedgc.) C. Moreau, C. coerulescens, and C. pluriannulata (Hedgc.) C. Moreau. However, these have more recently been collected on cut logs or stumps in forested areas. They are apparently common in the southeastern U. S. northward to New England and the Lake States area although more intensive collecting is needed. C. coerulescens (C. virescens as previously described) is fairly common in maple stands as the so-called "sap streak" but is commonly prevalent on fresh cut sapwood of many other hardwoods such as beech, oaks, and gums. C. fimbriata causes the canker stain of sycamore trees in the east. It has seldom been collected on wood surfaces otherwise. C. fimbriata was recently reported from cankers of aspen in Canada and the Lake States area. Its exact relationship to canker formation is not too well known.

Insect carriers of these hardwood species are not specifically known. Some work has been done in an attempt to determine carriers of the oak wilt fungus, C. fagacearum, but few attempts have been made with the other species except in the case of the Dutch elm disease.

SOME PROBLEMS FOR THE FUTURE

The studies so far have been mainly with the more common and widespread bark beetles such as Dendroctonus and Ips. Other minor bark beetles have also been collected but with only meagre information obtained. Several species of Ceratocystis have been reported in association with ambrosia beetles such as C. brevicollis Davidson with Trypodendron in aspen. Ambrosia beetles have frequently been encountered in conifer trees or logs in close association with bark beetles. In such cases the ordinary wood-staining fungi were also associated, confusing to the question of definite association. In the summer of 1963 isolations were made from galleries of an ambrosia beetle in a hemlock stump in New York state. The culture turned out to be a very striking species of Ceratocystis which is undoubtedly undescribed.

There are also a number of species complexes where intensive studies, both physiological and morphological, are needed. One of these is the C. minuta group where there are some morphological differences and probably physiological differences also. Another is the C. ips - C. montia - C. bicolor Davidson & Wells group. In this latter group there are instances of all-white perithecia and all-white cleistothecia.

Another is the C. coerulescens - C. virescens group in which case C. virescens Davidson is common only on hardwoods in the eastern states and C. coerulescens is common on Douglas fir sapwood in the northwest.

The Douglas fir fungus requires low temperature for growth in culture. Recently intermediate or other forms have been encountered which seem different in some morphological and cultural characteristics.

Additional studies are also needed to more clearly define the species limits or relationship between such species as C. pilifera, C. schrenkiana (Hedgc.) C. Moreau, and C. pluriannulata.

Besides these special species complexes much more information is needed for most of the species encountered. How can they be grown so as to insure good perithecial production? How can they be held in pure culture over long periods of time? Some species do not develop perithecia even after one summer in cold storage. Many will peter out over a period of three to ten years.

Another need is for fresh isolates of European described species for more accurate comparison with north american species. C. penicillata Grossmann was originally isolated from spruce in Germany. C. polonicum (Siem.) C. Moreau from Poland is another of the Leptographium - Verticicladiella group which we need for comparison.

It is hoped that the present study will provide a background for more intensive research in the future.

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SOME GYMNOSPORANGIUM SPECIES IN CALIFORNIA WITH SPECIAL REFERENCE TO GYMNOSPORANGIUM FUSCUM

Dan Y. Rosenberg

During the past half century, a number of species of Gymnosporangium have been observed on various hosts in California. In most instances, the occurrence of these various species was of little economic importance. Some of these species are:

1. Gymnosporangium harknessianum (E & E) on Amelanchier alnifolia (I) and on Juniperus occidentalis (III) (1).
2. Gymnosporangium inconspicuum on Amelanchier sp. (I) and Juniperus occidentalis (III) (2).
3. Gymnosporangium japonicum on Photinia arbutifolia (I) (2).
4. Gymnosporangium libocedri on Amelanchier alnifolia (Nott) I, Pyrus communis I, Crataegus douglasii (Lind.) III, and Libocedrus decurrens III (2).

During the examination by California state and county departments of agriculture personnel of various host material shipped into California from eastern states, Gymnosporangium juniperi-virginianae was intercepted on leaves and fruit of Malus spp. and on several different juniper species coming in on plants and cuttings.

In one instance juniper trees that originated in the midwest developed typical galls after having been grown in a Sonoma County nursery for one year. As a result of this discovery a quarantine was established to prohibit infected junipers from entering California.

G. libocedri has caused some damage to a commercial pear planting in the area around Placerville, California. In one instance, aecial infection of leaves and young fruit was heavy in the first two to three rows of the pear orchard adjacent to incense cedars. The infected pear trees were limited to an area of approximately 200 feet - in an arc or semi-circle from the southern margin of the orchard. Along this southern margin, several incense cedars bordered the pear planting. One of these large incense cedars was found to have several witches brooms scattered throughout the top. Examination of the affected branches revealed the telial stage of G. libocedri.

In the fall of 1960, Dr. A. H. McCain of the University of California reported to our Bureau the occurrence of the Gymnosporangium fuscum on pear in the area around Lafayette, California (3). Examination of the pear trees in the area revealed rust infection on 28 separate properties scattered over four square miles. Since this disease, considered to be very serious on pear in Europe, had never been reported in California or

in the United States, a quarantine was established to prevent the movement of infected juniper or pear outside of the four square mile area. During the early spring of 1961, the telial stage of G. fuscum was found on approximately 275 junipers (Juniperus sabina var. tamariscifolia and J. chinensis var. pfitzeriana) in the Lafayette area (4). An eradication program was initiated through the combined efforts of the Bureau of Plant Pathology and the Contra Costa County Department of Agriculture. This eradication program consisted of spraying all of the junipers in the quarantined area with a Cycloheximide preparation (Actidione) at 25 ppm and removing any infected junipers that were found. The spray program was initiated in the spring of 1961 and has continued every year. Intensive surveys are made of the pear trees in the early summer and fall for any aecial infection. Any infected trees that were found were sprayed with Actidione.

Several enlightening facts were brought out during our program. We were able to determine by means of bioassays that the Actidione remains in juniper in small amounts (.07 ppm) for at least six months. Actidione applied at the rate of 25 ppm will cause injury to junipers if applied when the air temperature reaches 80°F. The injury or phytotoxicity varied according to: 1) the growing condition of the plant (the plants with the greenest and most succulent growth were injured the most); and 2) the species of juniper involved. The "tam" juniper, J. sabina var. tamariscifolia, was the most tolerant to the Actidione of the junipers sprayed; the "pfitzer" juniper, J. chinensis var. pfitzeriana, was less tolerant and the hollywood juniper, J. chinensis var. torulosa was the least tolerant.

Since the first report of this rust in California, the eradicative measures undertaken (spraying with Actidione and removal of infected junipers) have resulted in rapid reduction of the disease. The Bureau of Plant Pathology and Contra Costa County Department of Agriculture plans to continue our spray program, our removal of infected junipers and our intensive survey of the area in order to eliminate the disease.

G. fuscum has been present in Canada, where it is now known by the European name, trellis rust, for several years but it was not recognized as G. fuscum until 1961 (5). Canadians are also attempting eradication. Shipments of junipers from Canada to the United States are banned by the United States Department of Agriculture because of the rust.

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SPECIAL REPORT

A. K. Parker

The presence in British Columbia of the red band disease of pines caused by Dothistroma pini Hulbary was confirmed in the spring of 1964. A survey revealed disease to be present in Vancouver Island plantations of P. radiata, P. muricata, P. pinaster, P. nigra var. calabrica, P. echinata x taeda, P. murrayana x banksiana, P. ponderosa and P. contorta. The disease was also found in natural stands of P. contorta on Vancouver Island. In the interior of the province the disease was found on a single P. ponderosa which had been planted outside its natural range and on P. contorta located in a natural stand.

Studies have been initiated to determine the range and life cycle of the pathogen in British Columbia, and the relative susceptibility of native pines.

APPENDIX I

NEW - ACTIVE PROJECTS

C. Cone, Seed, and Seedling Diseases

64-C-1 Seedling diseases of forest nurseries in Hawaii. (E. Trujillo)

The purpose of this project is to investigate the mechanisms of soil fumigation under tropical conditions. Also, to determine the pathogenic flora and possible mycorrhizal associations involved in the growth failure in first plantings. This project is done in cooperation with the Pacific Southwest Forest and Range Experiment Station.

D. Root and soil borne diseases or relationships.

64-D-1 The ecology of Phytophthora cinnamomi Rands. in forest soils. (E. Trujillo)

Emphasis will be to determine the distribution of the fungus in the Hawaiian forest. Immuno-fluorescent staining techniques will be attempted in order to study the pathogen in soil and to estimate its survival. This project is supported by a grant from the McIntire-Stennis cooperative forestry research program.

64-D-2 The host range of Armillaria mellea on subtropical species. (E. Trujillo)

Attempts are being made to determine susceptible and resistance in the different tropical and subtropical tree species used for reforestation in Hawaii. This project is supported by the Pacific Southwest Forest and Range Experiment Station.

E. Foliage diseases

64-E-1 Diseases of Christmas trees. (E. Trujillo)

This project was created to investigate the nature of the bronzing of the needles of Araucaria excelsa. Studies have shown this problem to be caused by light factors. The work is done in cooperation with the State of Hawaii Division of Forestry.

F. Stem diseases - malformations, witchs-brooms, dwarfmistletoes, etc.

64-F-1 Cytology and ontogeny of Arceuthobium douglasii, A. americana, and A. campylopodum f. laricis. (Clarence C. Gordon) (Int. Station and Montana State University Coop.)

Objectives: 1. To supply basic information required in applied research on methods to control these parasites in forest stands.

2. To find the stages in the cellular development of the parasites that might prove vulnerable to attack through control measures.

3. To supply cytological or anatomical information needed to classify dwarfmistletoe on a true phylogenetic basis.

4. To prepare a detailed illustrated (photomicrographs) monograph on the ontogeny of these three dwarfmistletoe species.

H. Stem Diseases - Rusts and Cankers

64-H-1 Derma dieback of Douglas-fir in the Cariboo Region, B. C. (A. Parker)

Five widely separated outbreaks of a severe dieback of Douglas-fir regeneration have been found in the Cariboo. The areas vary in size from a few hundred yards to several miles in diameter. All areas show the same symptoms and are associated with the same fungus, a species of Dermea (discomycete). An estimated 30 percent of the trees are affected in the severest outbreak.

64-H-2 Conifer stem rusts: pathological histology. (R. G. Krebill)

Objective: To learn the mechanism(s) of bark necrosis and manner of mycelial spread in Cronartium comandra and other species.

Miscellaneous Studies

64-K-1 The effects of Tuberculina maxima on white pine blister rust cankers. (James W. Kimmey)

Objectives: To collect factual data on the development and effects of naturally occurring T. maxima on blister rust cankers of western white pine, and to compare this with its development and effects on similar cankers treated with antibiotic materials.

64-K-2 The mode and time of infection of Tuberculina maxima on blister rust cankers of western white pine. (James W. Kimmey)

Objectives; To determine by inoculation at what stages in a blister rust canker's development it may become infected by T. maxima, and at what time of year infection takes place.

64-K-3 The time required for Tuberculina maxima to inactivate blister rust infections on western white pine. (James W. Kimmey)

Objectives: To determine the time required, after its establishment, for T. maxima to inactivate sporulation and growth of blister rust cankers of various sizes and ages on western white pine.

64-K-4 Diseases of Pinus lambertiana (H. Offord).

Objective: To identify and describe pathogens affecting sugar pine with special reference to the protection and management of this tree species under the impact of white pine blister rust.

APPENDIX II

TERMINATED PROJECTS

60-A- Dwarfmistletoe survey in western Montana. (D. P. Graham)

57-D-2 Trend of *Armillaria* infection on Pacific Silver fir. (G. Harvey).

61-E- Foliage diseases of western conifers. (B. D. Thyr) (Int. Station and Washington State University Coop.)

60-F-2 Translocation of natural substances and toxicants between mistletoes and their hosts. O. A. Leonard and R. J. Hull, Univ. Calif., Davis (as of July 1, 1964).

61-G-3 Study of decay in commercial conifers of the western white pine types. (O. K. Miller)

62-G-1 The response of heart rot fungi to extracts of host and nonhost wood. (H. H. Bynum)

63-G-1 The effect of holding temperature on apparent optimum temperature for growth of heart rot fungi. (L. A. Paine)

62-H-3 Epidemiology of *Cronartium comandrae*. (R. G. Krebill) I. Dissection plot studies. (Paper submitted to Jour. Forestry)

56-J-3 Deterioration of beetle-killed and wind-thrown Douglas-fir. (G. Harvey)

APPENDIX III

NEW OR MODIFIED TECHNIQUES

1. The use of Compressed Air in Root Excavations

L. C. WEIR

The study of root systems of forest trees in situ poses a number of problems related to excavation. Manual labour is time-consuming, difficult, and limits the number of systems that can be exposed during any period of time. Hydraulic equipment, as described by McMinn (1) saves time thereby increasing the amount of sample systems exposed, but is entirely dependent upon an adequate supply of water. Studies in Australia (2) have shown that compressed air would expose Armillaria infections on cultivated citrus trees but doubt was expressed as to its widespread application.

Materials.

The equipment consisted of a 125 cu. ft. Jaeger compressor capable of delivering 100 p.s.i., three 50 ft. lengths of hose, and a 5 ft. piece of pipe crimped at one end for a nozzle with a tap valve at the other end to regulate air flow. Tests were conducted both at Campbell River and on the Victoria watershed. The soil type was relatively dry sandy loam which shifted easily under the air jet, but the dense sub-surface mat of bracken and salal roots impeded the excavation somewhat.

Discussion.

The tests showed that root systems can be successfully excavated and that the time required was comparable with that taken with hydraulic equipment without the need for a water supply. Rocks up to 8" in diam. could be moved from the immediate vicinity by air flow and fine rootlets could be retained, complete with mycorrhizal nodules in some cases. With any technique of excavation the paramount problem is one of removing soil from the site. One method is to choose a site on a slope where gravity will minimize shovel work. The use of a movable conveyor belt would also aid in soil removal and might permit excavations by compressed air to be carried out on level ground.

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2. GROWTH OF FUNGI IN FOAM PLASTIC FOR USE IN PLANT INOCULATIONS

A. FUNK[1]

A simple technique for producing pre-packaged inoculum of filamentous fungi for testing pathogenicity has been developed. The fungi are grown in a layer of agar which impregnates the upper part of a porous polyurethane plastic pad. The pads are then applied directly to the host surface. In addition to quick, easy handling, the technique also provides improved conditions for the growth of the fungus and disease development. The technique has been used successfully in testing canker fungi of forest trees, but is adaptable to other fields of phytopathology.

Preparation

Pads of polyurethane foam plastic, approximately 3 x 2 x ½ inch are washed thoroughly in distilled water and pressed to near dryness. A small square approximately 1/2" x 1/2" and 1/8 inch deep is cut from the surface. Each pad is then placed in a petri dish and sterilized. Autoclaving does not affect the polyurethane.

Sterile, melted agar which has been cooled to the point of becoming slightly thickened is then added to the depression in the pad. This is best accomplished by pipetting a few drops at a time and allowing them to solidify. If too much agar is added at one time it may run through the pad. The agar is now inoculated with the test fungus which is allowed to develop until it penetrates the agar and becomes enmeshed in the plastic network.

The inoculum pads may be transported to the work site in the petri plates, or if desired, they may be wrapped in sterile paper and placed in plastic bags to prevent evaporation. Because the fungus culture is not easily damaged on the plastic pads, the latter method allows for easy transportation to difficult sites. In this form it may also be stored for considerable periods under refrigeration.

Application of Inoculum

The host surface is prepared in the same manner as in other techniques, making certain that an area larger than the pad is disinfected. The plastic pad containing the fungus culture is then applied face-down to the sterilized surface, and firmly bound to the host (Fig. 3). The pad is now covered with a clear cellulose film (Saran wrap) to prevent drying of the culture and also to protect it from wetting by rain or dew. Under greenhouse conditions where humidity is high and no danger of wetting exists, the inoculum pad need not be covered; the outer layer of the foam will keep the culture sterile.

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Discussion

Advantages in Handling.

1. Speed and ease of application. The prepared pads require no manipulation in the field and are applied with a minimum of exposure to contamination.

2. Storage and Transporting. Prepared inoculum may be stored until suitable conditions for inoculation occur and is easily transported when packaged. This is of particular importance when the period of host susceptibility is very short.

Advantages to Growth of Fungus.

1. Aeration and Light. With foam plastic the air is in contact with the culture at all times and light is able to penetrate also. Other techniques of inoculating usually employ wet cotton to maintain moisture, which frequently swamps the culture and also prevents light from penetrating.

2. Growth potential in the agar. The fungus is well established in the agar which is firmly held by the plastic mesh, providing nutrient for the fungus as it penetrates the host.

3. Moisture Control. By adjusting the covering of the inoculum pad the moisture content of the culture may be controlled.

4. Contact with the Host. The pliable nature of the inoculum pad enables it to conform to the contours of the host and make firm contact.

5. Exclusion of contaminants. The fine mesh of polyurethane foam plastic is able to exclude contaminating microorganisms from the air.

Disadvantages.

1. Some fungi appear to have an aversion to polyurethane and will not penetrate the pad.

2. Animals are sometimes attracted to the pads when used in the field and will tear them from the host.

APPENDIX IV

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APPENDIX V

MINUTES OF THE BUSINESS MEETING OF THE 12TH WIFDWC

The business meeting was called to order by Chairman Shea on Wednesday afternoon, October 14, 1964.

Secretary's Report: It was moved and seconded that the minutes of the previous meeting as written in the proceedings of the 11th WIFDWC be approved. The motion passed by a voice vote.

Treasurer's Report: The following report includes expenditures at the 12th WIFDWC and also additional costs incurred for publication of the proceedings. (R. F. Scharpf)

	Credit	Debit
Balance from 11th WIFDWC	$202.00	
Registration, Banquet	496.00	$401.70
Miscellaneous (coffee, goodies, proceedings)	44.60	52.60
Total	$742.60	$454.30
Balance (May 1965)	$288.30	

Mistletoe Committee Report: The report, circulated to the membership at the meeting, is included in Appendix VI.

Forest Disease-Recreation Committee: Report presented by H. R. Offord. It is a pleasure to report briefly today on the status of research and related activities in the field of disease-recreation.

The establishment of disease-recreation committee was approved at the 10th annual meeting, Victoria, B. C., in October 1962. Dr. C. G. Shaw, Chairman of the 1963 conference, appointed the below-named as the first committee of WIFDWC in this important matter.

In the absence of all three committee members, the first report of the disease-recreation committee was made by D. R. Miller at the 1963 conference at Jackson, Wyoming. His report was concerned chiefly with foresters' experiences in California with Willis Wagener's publication (PSW Station Res. Paper #1, 1963, "Judging Hazard from Native Trees in California Recreational Areas.")

In California there has been wide acceptance of the usefulness of these guidelines for judging tree hazards. Mr. John Mahoney of the National Park Service with the help of personnel of Yosemite and Sequoia National Parks first called attention to the need for some formal guidelines to aid in the recognition of high hazard trees and provided helpful case history data for Dr. Wagener's report. Interest in this report has been so widespread that it has been necessary to caution recipients of the publication that it was prepared for California tree species and does not necessarily apply to other regions and to tree species not covered in the guidelines.

In the past 2 years there has been a noticeable increase in forest research keyed to the ecology of campground vegetation. Also, there is a sustained interest in the problem of hazards from decayed and defec-

tive trees and windfalls, etc. Forest pathologists are clearly involved now in the recreation research and will continue to be needed for assistance in several phases of research having to do with recreational areas.

Another important development has been the shift in priority of disease research occasioned by the special requirements of land used for recreation. The impact of disease on the recreation resource presents a new framework of economics in which to judge the needs and costs of disease control. An example of the new look at disease research priority is the recently initiated project of Lee A. Paine (PSW Station, Berkeley) on "Heart rots of lodgepole pine in California recreational areas." In past years little work had been done in California on decays of lodgepole pine because it has not been a commercially valuable species. The maintenance of a large number of campgrounds located in lodgepole pine types in the Sierra Nevada puts a new and high priority on diseases of lodgepole pine.

We can expect that other conifers and hardwoods, in the past considered as unimportant or noncommercial species, will be included in research objectives as well as in further testing and improvement of guidelines for judging hazards from trees.

To a constantly increasing degree the development and protection of campgrounds and other intensively used recreation areas will involve the skills of forest pathologists and disease control specialists. This committee (not necessarily the same people) should continue to serve on behalf of WIFDWC to "keep pathologists in your campground." For the year ahead we invite notes on publications, new projects, case histories of hazardous trees, and other information in the field of the diseases and their impact on the management and protection of recreation areas.

R. E. Foster, G. M. Harvey, and H. R. Offord (Chairman)

Comments:

Shea also mentioned the increasing importance of disease-recreation problems. Recently a $25,000 suit was settled in favor of the plantiff against Weyerhaeuser Co.

Ziffer indicated the concern in the Lake States about hazardous trees in recreation areas.

Committee on Joint Pathology-Entomology Meeting:

Shea reporting, Wallis and Thomas absent.

Thomas attended the 1963 Entomology work conference, discussed the possibility of a joint meeting with the entomologists and after some deliberation of the matter with his committee put forth the following proposition: That WIFDWC should have a joint meeting with WIFIWC in the spring (March) of 1966 at Victoria, B. C. One day only should be

for joint meetings and the remainder of the week used for separate meetings.

W. W. Wagener suggested that one topic for discussion might be the "relationship between insects and stain fungi in fire killed timber." G. Hepting suggested that the "role of insects as vectors of decay fungi" would also be a possible topic for joint discussion. Further discussion and action on the proposal to have a joint meeting in 1966 is included under new business.

Committee on Ecology and Forest Diseases: R. Smith and E. Wicker.

Reports presented by R. Smith and E. Wicker. The committee indicated that detailed reports will be submitted to the secretary for inclusion in the proceedings. They emphasized the need for careful evaluation by the members for discussion next year.

The above reports of the committee are included as separates along with these proceedings.

Old Business: None.

New Business:

It was moved by R. V. Bega and seconded by S. Andrews that WIFDWC should have a joint meeting with WIFIWC in Victoria, B. C. in March 1966 and defer our fall meetings in 1965 and 1966.

After considerable discussion by Bega, Parker, Offord, and others the motion was defeated by a show of hands, 15 to 8.

The chairman thereby instructed the secretary to write a letter to Thomas informing him of the decision and suggesting that Thomas explore the possibility of having an exchange of men to serve on panels dealing with topics of mutual interest to both groups.

A resolution was made by Andrews and Hawksworth that: The classification "honorary member" be bestowed on members of the conference who retire from continuous employment in forest pathology. Further, that this policy be retroactive to include all past members of WIFDWC who have retired. Also that registration fees be paid by the conference for honorary members. Further benefits to be decided by the officers on an individual meeting basis.

A motion by Molnar to accept the resolution was seconded and passed by a voice vote.

The following resolution on indexing and retrieving forest disease literature was made by Offord:

WHEREAS the efficient coding, indexing, and retrieval of the world's literature in forest pathology is of vital concern and would greatly facilitate research in forest pathology, the Western Inter-

national Forest Disease Work Conference strongly endorses the objectives of INTREDIS (International tree disease register), IUFRO.

THEREFORE BE IT RESOLVED, That the Western International Forest Disease Work Conference approves of the INTREDIS project, and expresses the hope that Government and University research agencies and libraries will support this or similar projects of improved coding, indexing, and retrieval of forest pathology literature.

A motion was then made by Offord that WIFDWC endorse the above resolution. The motion was seconded and passed by a voice vote.

Place of the 13th WIFDWC:

Keen competition occurred in the selection of the meeting place in 1965. E. Trujillo extended a very tempting invitation for the group to meet in Honolulu, Hawaii. Paul Keener again made his strong pitch for the members to meet in Tucson, Arizona, the land of eternal sunshine and beauty. Alex Molnar, last but not least, invited us to meet in the lovely Okanagan Valley in either Vernon, Kelowna, or Penticton, B. C. Although our hearts may have been in Hawaii and our sympathy with Paul Keener for his gracious and persistent efforts, our budgets were the deciding influence for selecting "some place" in southern British Columbia for our next meeting.

Election of Officers:

R. V. Bega and Jack Bier were nominated for general chairman. It was then moved, seconded, and passed that nominations be closed. After some discussion of the candidates, Jack Bier was elected as chairman by a majority of the members present.

Stu Whitney was nominated for Secretary-Treasurer. It was then moved, seconded, and passed that the nominations be closed.

Adjournment:

On Friday afternoon, after hearty thanks to the program and local arrangements committees for their fine job, the chairman called for a motion to adjourn. The motion was made, seconded, and passed and the rush to return home began.

COMMITTEE REPORT ON STATUS AND NEEDS OF RESEARCH ON DWARF MISTLETOES

J. A. BARANYAY, F. G. HAWKSWORTH, C. L. QUICK, K. R. SHEA,

and J. R. PARMETER (Chairman)

Highlights of 1964 Research

I. Taxonomy, Hosts, and distribution

a. Seed of A. campylopodum from western larch, western hemlock, lodgepole pine (both coastal and interior forms), and white pine; A. americanum from interior lodgepole pine; and, A. douglasii from Douglas-fir were planted in October, 1963 on 5- to 6-year-old outplanted ponderosa pine, lodgepole pine, western larch, Douglas-fir (interior and coastal forms), white spruce, Engelmann spruce, Sitka spruce, western hemlock, white pine, grand fir, and Pacific silver fir. By March, 1964, about 21 percent of the over 2,600 seeds planted had been lost through rain, snow, etc. Of the remainder, 27 percent were classed as hollow, shrivelled or otherwise obviously not viable. Radicles had emerged from 3 percent of the seeds examined (Smith, Victoria).

b. Arceuthobium vaginatum f. cryptopodum was collected for the first time on Mexican white pine, Pinus strobiformis. A single infected tree, with two mistletoe plants, was found in an infected ponderosa pine stand on the Mescalero Apache Reservation, New Mexico. (Lightle, RM).

c. Field, laboratory, and herbarium studies are continuing toward a taxonomic revision of the genus Arceuthobium. For this revision, cytological, morphological, and anatomical evidence will be considered together with phenology, host relations, and geographic and ecologic distribution patterns. Field examinations indicate that some members of the A. campylopodum complex are indeed specific (e.g., f. divaricatum which is confined to Pinus edulis and P. monophylla) but others have a much broader host range than was previously thought (e.g., f. tsugensis, which in addition to tsuga, also occurs commonly on Pinus monticola, P. albicaulis, P. contorta, Abies lasiocarpa, and A. amabilis). (Hawksworth, RM, and Wiens, University of Colorado).

d. A manuscript on Arceuthobium in Mexico has been submitted to Brittonia. A total of 13 taxa are recognized. Of these, 3 (A. vaginatum f. vaginatum, A. verticilliflorum, and A. abietis-religiosae) were previously described from Mexico; 5 U. S. mistletoes (A. campylopodum formae campylopodum and blumeri, A. douglasii, A. vaginatum f. cryptopodum, and A. gillii) extend into northern Mexico; and 3 species and 2 subspecies are described as new. The genus is widely distributed in Mexico and at least 19 species of pines, as well as Pseudotsuga and Abies, are attacked. Despite the common occurrence of Arceuthobium in Mexico, the amount of damage they caused appears to be considerably less than in the western United States. (Hawksworth, RM, and Wiens, University of Colorado).

e. Dwarf mistletoe (A. campylopodum f. abietinum) was found to be common and very damaging to a stand of Brewers spruce on Flat Top Mountain, Siskiyou National Forest, west of Grants Pass, Oregon. (Graham, USFS Portland, Hawksworth, RM, and Wiens, University of Colorado).

II. Physiology and Anatomy

a. R. J. Hull completed certain aspects on the carbohydrates in the dwarf mistletoes and their hosts about April 1, 1964. The results of these findings may be found in his thesis "Studies on the Physiology of Mistletoe Parasitism (Arceuthobium and Phoradendron)", Botany Department, University of California, Davis. A portion of this has been accepted for publication in two papers in Plant Physiology.

Certain aspects of translocation of $P^{32}O_4$ have been investigated and reinvestigated since April 1. Also, the nutrition of host and green mistletoes on foliage-free lateral branches have been studied using both $C^{14}O_2$ and $P^{32}O_4$. The results largely confirm our previous findings that the nutrition of foliage-free green mistletoe infected lateral branches comes from other host branches and not from the green mistletoes; further, the green mistletoes receive only limited quantities of label from the defoliated branches.

Translocation in dwarf mistletoe infected Jeffrey pine, ponderosa pine, and red fir of C^{14}-labeled photosynthate conclusively demonstrated that the mistletoe infections do not block translocation in these species except under certain conditions. Physiological blocks do occur at times during the summer period due to the direction of the food materials into the mistletoes and the new tissues being formed on the infected branches. Such blocks were demonstrated to occur as a result of infection on a side branch, if defoliated. Such blocks are physiological rather than anatomical. (Leonard and Hull, U. C., Davis).

III. Life cycle studies

a. Studies have been continued through periodic observation of tagged infections and 1962 seeds on western hemlock at Lake Cowichan, Vancouver Island. An additional 100 seeds disseminated in 1963 were tagged. For information on persistence, 48 shoots were marked individually. Similar studies were extended to A. campylopodum on lodgepole pine near Horne Lake, Vancouver Island, and to A. campylopodum on western larch in southeastern British Columbia.

Seed traps placed in cleared areas around a single infected hemlock on Vancouver Island, and around an infected larch near Cranbrook are being examined twice monthly to determine approximate total seed discharge from the trees, period of dissemination, and quality of seed. (Smith, Victoria).

b. Initial seed velocities of four Colorado dwarf mistletoes were determined by high-speed photography. The results indicate average initial velocities of from 80 to 90 feet per second, or about twice that previously estimated by indirect means. (Hinds and Hawksworth, RM).

IV. Host-Parasite Relations - no reports

V. Effects on Hosts

a. Ring counts and measurements have been completed for 25 western hemlock with a range of infection intensity from light to severe. Five additional trees were felled in 1964 to augment some infection classes. Preliminary analyses show that the average height growth of severely infected trees was 19 feet for the period 1911-20, and only 7 feet for 1951-60. In contrast, the average height growth of lightly infected trees was 17 for 1911-20, and 14 for 1951-60 -- a reduction of only 3 feet. Data on volume and form are being processed. (Smith, Victoria).

b. Dwarf mistletoe and its effect on growth and mortality in lodgepole pine stands of Alberta. Data have been processed for publication. (Baranyay, Calgary).

c. The ponderosa pine growth impact data are still being analyzed. One of the main reasons for delay is the difficulty of getting good determinations of mistletoe effects on height growth. In general, results of the study appear to be about as expected: light infection (i.e., in lower crowns only) has little effect, but heavy infection increases mortality rates several-fold and reduces volume growth by a third or more. (PNFRES, Portland).

d. Diameter increment of dwarf mistletoe infected ponderosa pine (Keen's-age-class 2) before and after logging was investigated in south-central Oregon. Growth of severely infected trees was significantly less than that of trees in all other infection class both prior to and after logging. The ratio of growth of severely infected trees to that of all others remained approximately the same throughout. (1:1.7) All trees, however, responded favorably to release. (Shea, Weyerhaeuser Co.).

e. Diameter increment of ponderosa pines in Keen's-age-classes 1 through 3 was significantly reduced by severe (rating of 6) dwarf mistletoe infections but not by lighter infections. Natural tree to tree variation may mask effects of lighter infections. Brooming was associated with greater reductions in growth than was comparable levels of infection without brooming. Height growth apparently is affected more adversely by dwarf mistletoe than is diameter increment at least in young trees. (Shea, Weyerheauser Co.).

VI. Ecology

a. Efforts to pinpoint the northern limits of Arceuthobium vaginatum f. cryptopodum were continued. The northernmost known shoot of this dwarf mistletoe occurs in northern Larimer County, Colorado, about eight miles south of the Wyoming boundary. Studies of the factors influencing this northern limit are planned. (Hawksworth, RM).

VII. Control-chemical

a. A chemical applied to Douglas fir in 1960 by mist blower has continued to provide control of dwarf mistletoe. The success of the material appears to be related to season of application and the method used. Considerable investigation is still required. (Shea and Rediske, Weyerhaeuser Co.).

VII. Control-chemical - continued

b. See also: Quick, C. R. Experimental herbicidal control of dwarf mistletoe on some California conifers, U.S.F.S., P.S.W. Research Note 47, 9 p., 1964.

VIII. Control - Biological

a. Colletrotrichum gloesgsporioides, originally collected and described on dwarf mistletoe on red fir in California has been found on several other species and forms of dwarf mistletoe. In California it also occurs on Arceuthobium americanum and on A. campylopodum f. campylopodum. It has been collected on A. campylopodum f. divaricatum in Colorado and New Mexico and occurs on A. campylopodum f. microcarpum in Arizona. Although all isolates have not been tested some were found to cross infect other mistletoe forms or species. (Scharpf, P.S.W.).

IX. Control - silvicultural

a. Some of our results suggest that rigorous sanitation, to reduce mistletoe populations to very low levels, may be un-economic--that, in young stands making good growth, mistletoe may not reduce final yields by more than a few percent even if a cheap job of sanitation results in a moderate level of infection. The most interesting consequence of this hypothesis, thus far, is the healthy spirit of controversy it has evoked among mistletoe researchers. (PNFRES, Portland).

X. Surveys

a. The dwarf mistletoe infected lodgepole pine stands, reported last year, were air photographed again this year using infrared camouflage detection film, the films are under processing. (Baranyay, Calgary).

XI. Miscellaneous

a. "CAN-YOU-TOP-THIS?" Department. Arceuthobium americanum was found at an altitude of 11,000 feet on Monarch Pass, Colorado. Is this the highest American Arceuthobium north of Mexico? (R. C. Thobium, RM).

Needed Research

I. Taxonomy, hosts, and distribution - no suggestions.

II. Anatomy

It would be interesting to know something about the details of the anatomical association between the living cells of mistletoes and their hosts (dwarf and green), studied using an electron microscope. (Leonard and Hull, U. C. Davis).

III. Life cycles - no suggestions

IV. Host-Parasite relations - no suggestions

V. Effects on hosts

a. We need more precise measurements of growth impacts of various levels of infection in thinned stands. Because of the multiplicity of variables affecting tree growth, and the impossibility of determining past trends of infection accurately from present evidence, such measurements will require long-term studies on numerous plots. A few plots have been established but many more are needed. (PNFRES, Portland).

VI. Ecology

a. Research is needed to study the phenology of dwarf mistletoe flowering and to determine if there are differences in flowering and seed production between recognizable forest associations or sites in Alberta. (Muir, Calgary).

b. To study the development of the systematic habit of Arceuthobium and the factors influencing broom formation. (Muir, Calgary).

VII, VIII, IX. Control

a. Studies should be continued on all aspects of control. Some approaches towards chemical control are suggested from the tracer and other studies. However, eventual success can best be achieved through an open-minded or flexible approach to the problem.

X. Surveys - no suggestions

XI. Miscellaneous - epidemiology

a. We also need a much stronger basis of data on rates of increase of mistletoe populations, spread from tree to tree, and movement upward in the crowns. This information will eventually be provided by studies on permanent plots. In the meantime, to meet urgent present needs, additional analyses should be made of past infectio trends by detailed dissections of trees and infections on temporary plots. (PNFRES, Portland).