Regional Research Project Number W-187

Interactions Among Bark Beetles, Pathogens, And Conifers in North American Forests

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PROJECT NUMBER: W-187

Title: INTERACTIONS AMONG BARK BEETLES, PATHOGENS, AND CONIFERS IN NORTH AMERICAN FORESTS

DURATION: October 1, 1998 to September 30, 2003

STATEMENT OF THE PROBLEM:

Bark beetles and pathogens interact to cause extensive losses in the forests of North America. The vast areas affected, the hidden nature of root diseases, the episodic nature of bark beetle infestations and the declining emphasis on extracting timber contribute to a lack of current, regional estimates of the impacts of these pests. Yet these losses are significant. For example, in 1997 an aerial survey of National Forests in California found groups of mortality on more than 90,000 acres (table 1). While the exact cause of mortality was not determined, bark beetles and root diseases are the most likely candidates. These numbers are very low compared with most years, as moisture was above normal for several years prior to 1997.

Table 1. Tree mortality within the national forest system, California --1997[a]. Source: Forest Pest Conditions In California, 1997. (http://www.r5.pswfs.gov/fpm/cond97/forpest97.htm)

	Pine Mortality			Fir Mortality		
Locale	Acres	Volume	Number of trees	Acres	Volume	Number of trees
Northern California	67,955	38.30	217,561	10,791	25.78	115,308
Cascade/Northern Sierra	4,193	2.39	49,641	3,111	3.33	25,188
Central/Southern Sierra	4,111	6.12	52,672	257	0.32	2,681
Southern California	1,884	0.02	5,633	145	0.00 b	602
TOTALS	78,143	46.83	325,507	14,304	29.43	134,802

a All volumes are in millions of board feet.

b Trace; did not register with computer program

Pitch canker provides yet another example of the extent of insect-pathogen problems. This introduced pest was found in California in 1986. After just 14 years, an active infestation zone of 23.1 million acres was declared (http://frap.cdf.ca.gov/pitch_canker/zone_infestation.html). Movement of potential host materials out of this zone is discouraged.

There are far too many insect-pathogen-host systems for entomologists and pathologists to study each one. W-187 and its predecessor W-110 have made considerable progress by developing a general model of potential interactions, and studying the specific interaction, and then fitting the results into the context of the larger model. This has permitted us to extrapolate our findings to other insect-pathogen systems, and has greatly increased our understanding of all of our pest systems.

JUSTIFICATION:

Regional research project W-110, and its successor W-187 have solved problems facing our forests, but the changing management objectives from wood products to other resource uses (including wildlife and resource protection), the dramatic increase of urbanization in our native forests over the last two decades, and the increased threat of introduced pests require new approaches. New scientific technologies enable us to address old problems that were previously intractable. As outlined below, regional research project W-187 should continue because of the values at stake, the intrinsic nature of the problems, the need for cooperative work, the benefits that accrue from the proposed research, the relationship to current regional and national priorities, and this project's impact on science. An important value of the collaborative research proposed here is that the ecological mechanisms associated with interactions among bark beetles, pathogens and conifers are similar among different taxa found throughout North America. The investigators participating in this regional project present strengths through a diversity in research approaches, individual subject species, and by research projects conducted under different climatic regimes.

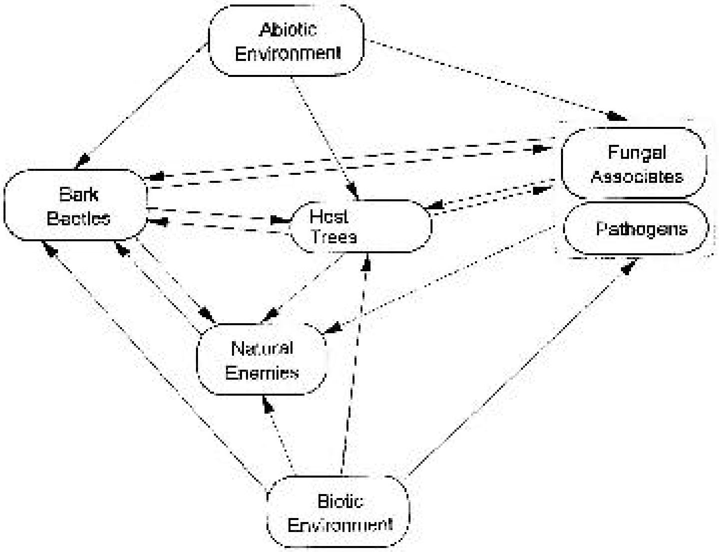
Values: The commodity value of western forests has supported the economy of many parts of western North America. However, society also values forests for watershed, wilderness, recreation, and habitat for threatened and endangered fish and wildlife species. These ecological and aesthetic values can exceed those of forest products such as timber, and their consideration has caused the removal of many forests from the timber-producing base. Traditional methods of pest management often rely on silvicultural options - most of which involve some form of timber harvest or regeneration techniques to modify the environment. When forest lands are reserved, these silvicultural options are restricted or severely limited. Yet these valuable lands are what must be managed to prevent ecological imbalances that can cause devastating outbreaks of pests and loss of the resource, as well as the quality life for the largely rural communities dependant on the forest. As forests are removed from the timber-producing base, the value of the remaining timber-producing forests will increase. Pest management in these forests will thus become increasingly important. Also, with Ecosystem Management, we need to more fully understand the role insects and pathogens play as disturbance agents in healthy, functioning ecosystems, and the effect of management on these processes.

Bark beetles and root pathogens are among the most important agents that destroy forests. In 1991, the Southern pine beetle was at outbreak levels in over 10.7 million acres in eight mid-Atlantic and southern states (Hofacker, et al., 1992). In the same year, over 2.2 million trees were killed by the mountain pine beetle in 11 western states. Losses due to the Douglas fir beetle, western pine beetle, spruce beetle and the fir engraver beetle occurred on 735,000 acres in Oregon and Washington. Root diseases are responsible for approximately 18% of the total tree mortality in the western United States, causing losses of up to 1.5 billion dollars annually (including stumpage worth) (Smith, 1984). These losses are greatly underestimated since they include only losses due to tree mortality, while losses caused by growth reduction are probably much greater, but are very difficult to measure. Perhaps more important, these are losses only in timber value; watershed, wilderness, recreation, and habitat values are not considered.

Importance and Extent of the Problem: Losses due to pests are extensive. Reducing these losses can provide a more stable supply of timber and additional social benefits. Native insects and diseases severely debilitate forests, leading to catastrophes, such as the Yellowstone fires which burned forests that were heavily infested by mountain pine beetle, dwarf mistletoes, and root diseases. Unfortunately, much of our understanding of insects and diseases comes from studies of pests in outbreak status. We must know much more about the ecological interrelations among insects, diseases, symbiotic organisms, and trees in non-epidemic situations. W-187 is focused on the essential components of complex tree-beetle-fungal interactions so that we can better understand forest and ecosystem level processes.

Need for Cooperative Research: The task facing this group is large and too complex for any one group or discipline. Multiple disciplinary cooperative research is needed to assemble the expertise required to attack this problem. Entomologists, pathologists, systematists, and physiologists working under the aegis of W-187 have already made significant progress toward understanding the role of bark beetle-carried fungi in triggering host wound response and tree decline. We developed a conceptual model of host tree - bark beetle - fungus interactions (Fig. 1). This model, in addition to visualizing our concepts, serves as an operational framework for our collaborative research. Work on one process or interaction, without consideration of other processes, has applicability only to that population at that time. For example, studies of bark beetle brood success under various conditions provide little useful information unless they consider the taxonomy and biology of beetle fungal associates. By fitting our work within the context of

Figure 1. Conceptual model of bark beetle-fungus-tree systems.



this conceptual framework, and sharing our techniques, research results can be extrapolated to fill gaps in our knowledge. For example, we are studying the response of Clerid predators to the pheromones of their host bark beetles in three different geographic settings, and in three beetle-tree systems. These predators are more abundant the year following the peak of the bark beetle population. This ultimately leads to development of principles which can be further evaluated to determine their broad application, for example that predator populations depend on host density. Similarly, cooperative research will also help workers overcome the taxonomic barriers that often prevent them from correctly identifying subtle differences among fungal taxa. By fitting our research into this conceptual framework, we eliminate fragmented research and wasteful duplication and broaden the expertise and methods available to solve the problems.

Benefits: W-187 has benefitted forestry throughout the country in many ways. Resource managers already use knowledge provided by W-187 scientists regarding strain variation and the correlated host specificity in *Heterobasidion annosum, Armillaria ostoyae* and *Leptographium wageneri* in the management of these root diseases. W-187 scientists have produced the research that lead to the major reclassification of Ophiostomatoid fungi associated with bark beetles. Similar recognition of strain differences among fungi and determination of the complex taxonomic relationships between fungi associated with bark beetles have provided insights into the differences in host colonization and geographic distinctions in the biologies of broadly distributed species. W-187 scientists have also begun to unravel the complex interaction between bark beetles, their semiochemicals, fungal associates, and natural enemies. This understanding will help resource managers to minimize pest populations, especially in areas where harvesting is not an option.

The results from the research efforts have provided both management tools and fundamental understanding of complex relationships among pathogenic fungi, bark beetles, and host trees. The biotic interactions have been studied within the context of the abiotic environment which facilitates extrapolation of the conclusions to different systems in different regions. The ability to extend both the fundamental and applied information across different systems is one of the key benefits of the a regional research program of this breadth.

RELATED CURRENT AND PREVIOUS WORK:

A search of the CRIS database turned up 126 projects with the keywords forest and insect or forest and disease. Of these, 17 were relevant to W-187, and all but three institutions were members of W-187. These people will be contacted shortly. Two projects which are not part of W-187 are seeking to understand the biochemistry of host defenses. Both PI's are biochemists, and may be unaware of W-187. Studies of host biochemistry are a missing component of W-187 efforts, but for results to be meaningful, they must be placed in the larger context of host stress, host volatile production and potential host-induced resistance.

Only one regional project, W-189 Natural Products Chemistry as a Resource for Biorational Methods of Insect Control, is even closely related to W-187. Their objectives focus on insect and host plant physiology. The CRIS report for this project was missing, so we could not identify the host plants of interest. We will contact this group to determine if there is any common ground.

Most of the work in North America on interactions among bark beetles, pathogens and conifer hosts is done by members of our group. This work was extensively reviewed in a 1993 book entitled "Beetle-Pathogen Interactions in Conifer Forests" (Schowalter and Filip 1993.) This book remains the key reference in this area. The Critical Review appended to this proposal discussed our more recent publications dealing with specific host-insect-fungus systems.

The interaction between entomologists and pathologists continues to provide a unique perspective and approach for solving problems and synthesizing progress. W-187 members Paine, Raffa and Harrington collaborated on an annual review article entitled "Interactions among scolytid bark beetles, their associated fungi, and host conifers" (Paine et al 1997). They cite three key critical areas in bark beetle-fungus-host tree relationships where better understanding is needed:

C characterization of the multiplicity of potential interactions among organisms
C description of the dynamic rate of interactions at the biochemical level; and
C examination of a broader taxonomic range of associated microorganisms.

Each of these needs is covered under at least one of W-187's objectives. W-187 brings together the scientists working in this area, provides a framework for fitting their research results into a larger conceptual model, and facilitates the collaboration necessary to understand the complex problem of bark beetle-pathogen-conifer interactions.

OBJECTIVES:

1. Characterize the role of biotic and abiotic factors in predisposing trees to bark beetle attack and subsequent mortality

2. Characterize interactions among conifer hosts, bark beetles, their natural enemies, and vectored fungi.

3. Characterize the taxonomic diversity and genetic structure of key fungal pathogens and symbiotic fungi associated with insects on North American conifers.

PROCEDURES:

Included with the procedures for each objective are the cooperative studies to be undertaken in this regional project. Since many of the interactions are shared in objective 1 and Objective 2 (see Fig. 2 and 3), these shared interactions are discussed together. The procedures for objective 3 are discussed separately. It should be noted that other research planned by the participants often extends beyond the cooperative research outlined in this regional project description.

Objectives 1. Characterize the role of biotic and abiotic factors in predisposing trees to bark beetle attack and subsequent mortality; and **2.** Characterize interactions among conifer hosts, bark beetles, their natural enemies, and vectored fungi.

Interaction 1: Effects of the abiotic environment on bark beetles.

Studies are proposed to determine the effects of abiotic conditions, particularly temperature influence on bark beetle flight period activity, host colonization, developmental success. Previous studies have examined the survival of larvae under winter conditions and how aspect and snow cover affect the developmental rates and mortality. These field and laboratory studies on cold hardening will be continued and expanded to other host tree systems. The results can be incorporated into beetle phenology and population growth models to refine their predictive values.

Interaction 2: Effects of the abiotic environment on host trees.

Water availability, temperature, ozone, fire, and soil characteristics may change tree resistance to bark beetle colonization, susceptibility to fungal infections, or the quality of the tree as a resource for beetle development. Characteristics of host resistance or suitability, including photosynthetic rate, stomatal conductance, terminal and radial growth, resin flow, carbohydrates, phloem thickness, resin composition, and volatile emission, will be evaluated following experimental manipulation of the critical abiotic factors. Drought, in particular, may lower the inducible defense systems that some plants possess. Atmospheric pollutants, including ozone and dry nitrogen deposition, may alter the source-sink relationships within trees and change the allocation of photosynthate between growth and defense within trees.

Interaction 3: Effects of the abiotic environment on fungal pathogens and fungal associates.

Fungal pathogen populations are greater in environments that are stressful to their hosts. The extent of *Heterobasidion annosum*, *Armillaria ostoyae,* and *Leptographium wageneri* pathogenicity across managed and unmanaged forests will be related to habitat type that incorporates detailed site information, such as soil type, fertility, drainage, drought, defoliation history, and other diseases. The research will provide information to explain how host stress operates to increase the incidence and rate of infection and mortality caused by *A. ostovae*. In addition to studies on root pathogens, similar research will be initiated to examine temperature dependent growth rates of the fungi associated with bark beetles in the host tissue.

Figure 2. Interactions diagram for Objective 1 with participants. Numbers refer to the interaction being studied.

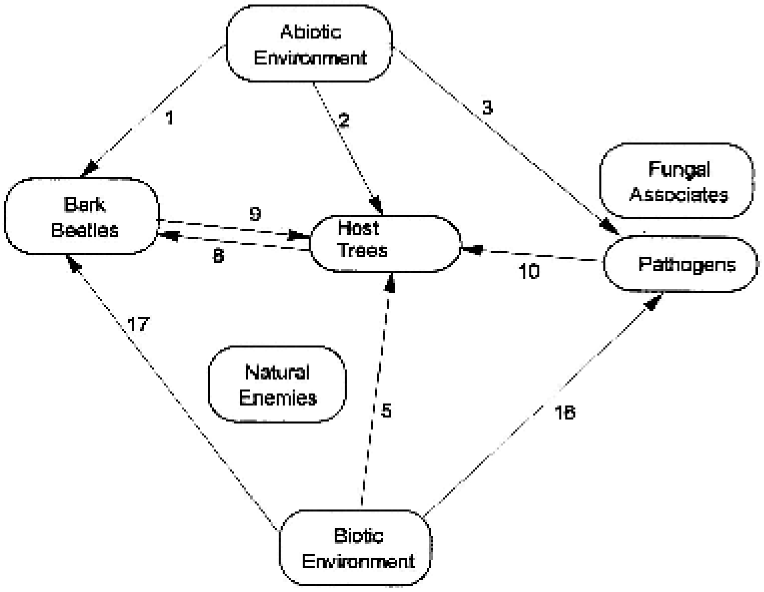
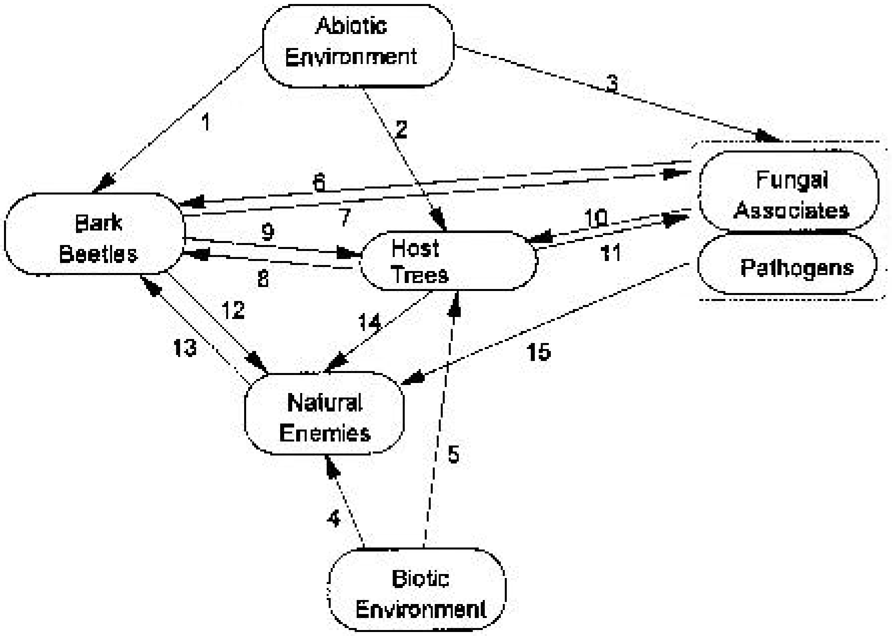


Figure 3. Interactions diagram for Objective 2 . Numbered arrows refer to the specific interaction being studied.



Interaction 4: Biotic environmental effects on the natural enemies of bark beetles.

Research will continue in several bark beetle- natural enemy systems to determine the influence of various biotic factors such as other beetle species as alternate hosts or nutrition on natural enemy populations. Studies are planned to evaluate the effect of predators on populations of different beetle species, the response of natural enemies to components of the beetle host pheromones, how these responses change across broad geographic areas, the influence of the fungi associated with the beetles on the natural enemies, and the influence of host trees on the interactions between natural enemies and their bark beetle prey.

Interaction 5: Effects of the biotic environment on host trees.

Biotic factors such as defoliators, mistletoes, rusts, and intraspecific competition among trees can affect the suitability of host trees for bark beetles and associated fungi. Defoliators, parasites, pathogens, and plant competition can affect photosynthetic rates, stomatal conductance, terminal and radial growth, secondary metabolism, resin flow, and phloem chemistry (especially carbohydrates, nitrogen, and the capacity for inducible defenses). When entire stands are affected, as in spruce budworm outbreaks or thinning practices, there is great potential for landscape level effects on bark beetle population dynamics.

Interaction 6: Effects of fungal associates on bark beetles.

Fungal associates specifically vectored within elaborate mycangia, commonly associated with the external surface of the insect body, or on phoretic mites appear to influence bark beetle success by improving nutritional quality of the host, limiting the growth of antagonistic fungi, or by limiting the impact of host defenses on attacking adults. The nature of these interactions are of critical ecological interest but not well understood. The almost universal association of *Dendroctonus* spp. with fungi and the apparent coevolution of the specific mycangial relationships suggest that these associations are critical for both beetle and fungal success.

Interaction 7: Effects of bark beetles on fungal associates.

Bark beetles may affect the success of their fungal associates through the process of transmission into the host trees, by influencing the growth of the fungi in the tree, or by fostering the growth of the fungi within the mycangia. The fungi are acquired by the adult beetles prior to emergence from their natal host and must be maintained between hosts. This is accomplished by many *Dendroctonus* spp. within a mycangium. Secretory cells lining these structures in many beetle species apparently foster the growth of the fungi such that there are fungal propagules present during the colonization process. In addition, there may be by-products of insect metabolism (e.g. nitrogenous waste) that stimulate fungal growth and sporulation. Because these conidia may be important sources of insect nutrition,the interactions of the beetle on the fungi may be critical for insect success.

Interaction 8: Effects of host trees on bark beetles.

Bark beetle reproductive success is affected by host tree condition. The ability of the tree to resist colonization (especially as influenced by resin flow and lesion formation) may result in increased adult mortality and limit the fecundity of surviving bark beetle adults. In addition, phloem characteristics such as thickness, moisture content, carbohydrate levels, and nitrogen content, may impact the survival, development time, and adult size of bark beetle progeny. Correlating components of beetle success (number of successful and failed attacks; number of galleries; gallery length; number of eggs; number of larvae, pupae, and emerging adults) with tree characteristics will clarify our understanding of host effects on beetle population dynamics.

Interaction 9: Effects of bark beetle populations on host trees.

The effects of the host tree on bark beetle populations is the culmination of the entire conceptual model. All the other interactions describe factors which lead to successful bark beetle colonization and subsequent tree mortality, whereas this interaction is the final result. All the other interactions ultimately have an effect on this one. Colonization by the beetles results in tree debilitation either through exhausting nutritional reserves or primary resin defenses (resin flow rate and volume of flow), through transmission of pathogenic fungi or through a combination of these mechanisms. A consequence of tree mortality is resource availability for many species of beetles which may result in a population increase or a population refuge. Area wide population levels can be evaluated through permanent plots or periodic surveys and spot growth models.

Host trees partially infested with less aggressive bark beetle populations provide a refuge for endemic populations of the more aggressive bark beetles. Secondary bark beetles can predispose host trees to the more aggressive bark beetles. For example, at endemic mountain pine beetle population levels, trees infested with less aggressive secondary beetle species provide a refuge for the mountain pine beetle, thereby allowing them to maintain an endemic state. Diseased host trees play a role in providing a refuge for endsemic beetle populations.

Interaction 10: Effects of pathogens and fungal associates on host trees.

Colonization of root systems by pathogens is an important contributing factor in successful bark beetle colonization. The degree of infestation by root pathogens for successful and unsuccessful colonization remains unclear and will be the subject of continued investigations to assess individual tree and stand risk. Percent infection can be related to changes in the physiological processes that directly impact bark beetle survival immediately following initial attack. Preliminary evidence suggests that Armillaria root disease and infection by Leptographium spp. can alter the composition of host resins and induce moisture stress, both processes of which are important in the preformed defense system of trees. Similarly, the fungi vectored by bark beetles may reduce tree vigor, reduce the host resistance, and may result in tree mortality. The fungi may alter water conduction through the stem or affect the preformed (resin flow or volume of flow) or induced components of host resistance. The impacts of the fungi on the tree may directly affect beetle colonization success. In addition, the level of twig and branch mortality caused by the pitch canker fungus and *Pityophthorus* spp. is related to the occurrence of bole cankers and subsequent tree mortality caused by *Ips*.

Interaction 11: Host tree influences on fungal associates of bark beetles and tree pathogens.

The host tree species may influence the species composition of the fungal associates of bark beetles as well as the pathogen species. In addition, there may be seasonal effects expressed through tree phenology and tree chemistry on this interaction. Other interactions with the host tree that may influence fungi are drought stress, shade stress, elevated temperature and elevated ozone levels as well as other factors that may affect host tree condition.

Interaction 12: Bark beetle influences on natural enemies.

Natural enemies of bark beetles show a density dependent response to bark beetle populations. Some aspects to be considered in this interaction are the influence of bark beetle species and the density of natural enemies, i.e. some beetle species are more heavily attacked by natural enemies. In addition, natural enemy complexes may vary due to bark beetle species or to geographic variations within a species. This will be tested through both surveys and responses to pheromone lures.

Interaction 13: Natural enemy influences on bark beetles.

Although there have only been a few attempts to demonstrate the impact of natural enemies on bark beetles, many researchers feel that parasitoids and predators regulate bark beetle populations. The assumptions can be tested in many different systems in laboratory cages, on artificially infested bolts, or by exposure of beetle brood in cut trees. Beetle densities will be recorded by rearing and final peeling and counting the life-stages still beneath the bark. Natural enemies will be evaluated in the same way.

Interaction 14: Influences of host trees on natural enemies of bark beetle.

Host tree species, as well as host tree condition, may affect the natural enemy complexes of a bark beetle. The natural enemy complex of bark beetle species that infest different hosts may be compared by rearing cut infested bolts. Both predators and parasitoids are collected from specialized rearing containers, identified, sexed and counted.

Interaction 15: Bark beetle fungal associates and tree pathogen interactions with bark beetle natural enemies.

Studies in Georgia and California have indicated that bark beetle parasitoids may be using volatiles from logs infected with bark beetle fungal symbionts to locate their prey. However, the question of whether bark beetle natural enemies are capable of vectoring tree pathogens remains unclear. For example, there are a large number of cortical-feeding insects that have been demonstrated to vector the pitch canker fungus, but further research is required to determine whether natural enemies emerge from infested material carrying fungal spores and whether the natural enemies are capable of fungal transmission.

Interaction 16: Effects of biotic environment on pathogens and fungal associates.

The biotic environment, which includes the population dynamics of living organisms, can affect the epidemiology of pathogens/fungal associates and therefore affect disease severity in forest ecosystems. Pathogen populations and disease severity may be greater in unmanaged forests or improperly managed forests. In addition, living organisms such as bark beetles, defoliating insects, and fungi affect their host trees and indirectly affect pathogens and fungal associates. For example, the incidence and severity of *Armillaria* spp. may be greater where the fungal anatagonist *Trichoderma citrinoviride* is absent. In addition, the interaction between the biotic (e.g., fungal pathogens, defoliators) and the abiotic factors (e.g., drought) may be critical in pathogen success. Biotic agents such as fungi or insects directly affect pathogens and fungal associates through direct attack or feeding (i.e., insect feeding on dwarf mistletoe plants).

Interaction 17: Effect of the biotic environment on bark beetle populations.

There is no question that the biotic environment influences the dynamics of bark beetle populations. The biotic environment continues to be represented in our model by many attributes, including landscape level processes and patterns, forest ecosystem processes (e.g. nutrient cycling, animal interactions), stand structure, management, and fire effects. Typical features of the ecosystem such as the ones listed are hypothesized to be important drivers of bark beetle populations in particular. Recent technological advancements in tools such as remote sensing and geographic information systems, as well as canopy patch dynamics techniques, are providing a means to study the response of beetle and pathogen populations in the landscape context. The influences of management on these interactions is being examined by comparing patch profiles in wilderness areas with previously harvested sites nearby. How the spatial/temporal aspects of bark beetle populations and host trees infested with the root diseases interact to predispose trees to mortality is also being explored.

Objective 3: Characterize the taxonomic diversity and genetic structure of key fungal pathogens and symbiotic fungi associated with insects on North American conifers.

Although significant progress has been made, the fungi associated with bark beetles have long presented taxonomic problems. These fungi all have morphological features, including evanescent asci and long necked perithecia that facilitate arthropod dispersal. Although genera such as *Ophiostoma*, *Ceratocystis, Leptographium,* and *Ceratocystiopsis* have traditionally been placed in a single taxonomic order or even family, there is increasing evidence that the group is polyphyletic. Species of *Ceratocystis* represent a separate convergent lineage. Other scolytid associates, species of *Ambrosiella,* do not even constitute a monophyletic genus, but rather have some species related to *Ceratocystis* and others to *Ophiostoma*. New molecular techniques and cladistic analysis provide a means to begin to determine relationships of fungi with convergent morphological characters.

Defining monophyletic groups is an essential first step toward the eventual goal of this part of the project, the development of rapid identification protocols for species identification and determination of population structure. Once conservative DNA regions have provided evidence of monophyletic groups at the ordinal and generic level, elucidation of inter- and intraspecies level problems can begin using more variable regions of the DNA. Identification of fungal taxa will be accomplished using standard morphological criteria, vegetative compatibility, and nucleotide sequence data where available. Monophyletic groupings of fungal taxa will be based on cladistic analysis of DNA sequence data from appropriate regions in the nuclear or mitochondrial genome. The identification of monophyletic groups by sequence analysis will facilitate the development of taxon-specific oligonucleotide probes, which in turn will enable rapid identification of difficult species. A similar approach has been used to identify ectomycorrhizal fungi. Virulence and host range variants will be circumscribed using standard greenhouse and field assays for pathogenicity. To characterize population structure, variants will be identified based on allozymes, restriction fragment length polymorphisms, randomly amplified DNA, or direct measures of DNA sequence divergence. These data will be analyzed using standard methods in population genetics to describe population structure and test for populations subdivision. A diagram of the four levels of activity and their relationship is shown in Fig. 5.

EXPECTED OUTCOMES:

The proposed research is a continuation of a long and highly productive research effort. Previous research conducted under the auspices of Regional Research Project W-187 and its predecessor W-110 has changed the management of root pathogens and bark beetles throughout western North America. The results have provided basic understanding of the chemical communication among insects, the relationships between the beetles and vectored fungi, and the risk of tree mortality that is a function of the interactions between beetles, host tree condition, and pathogenic fungi. The reserach has had a significant impact on forest management on a local and a landscape scale. It is exspected that the proposed research will have outcomes that build on the fundamental and applied knowledge base that has been accumulated. Research results will produce significant contributions to the scientific literature. In addition, the results will be incorporated into forest management practices which address the interactions among the insects, fungal pathogens, and host trees to produce healthy, environmentally sustainable, and productive forests for the future. Research results will be communicated to a variety of audiences through scientific publications, popular articles, book chapters, scientific meetings, and extension programs. Unlike agricultural fiber production, forest fiber has both environmental and commercial value that must be maintained over a long growth cycle. The environmental values are particularly important and must be balanced with the values for natural resource utiliztion. The research results generated from the proposed project, both fundamental and applied, will be particularly important for management of this valuable natural resource over the long term to meet both enviromental anr economic goals.

ORGANIZATION:

The technical committee shall consist of the Administrative Advisor (non-voting), and at least one representative from each participating Experiment Station and Cooperating Agency. Each participating Experiment Station and Agency shall be entitled to one vote.

The officers of the Technical Committee will be a Chair, Past-Chair, and a Secretary. Terms of office will be one year. The Chair, Secretary, and the Past-Chair will constitute the Executive Committee, which will be responsible for the routine affairs of the Committee, and will pass on pressing matters which arise between annual meetings of the Technical Committee. The Secretary will become the Chair in the subsequent year and a new Secretary the elected each year at the annual meeting with all members of the Technical Committee eligible for office. In addition, three members-at-large will serve for a period of two years. These positions are staggered so that a member-at-large is elected by the Technical Committee each year. Along with the officers, this constitutes the Coordinating Committee for the Technical Committee.

The Chair, in consultation with the Administrative Advisor, will notify the Technical Committee members of the time and place of the annual meeting, prepare the agenda, and preside at the meeting. The Secretary will be responsible for the final preparation and submission of the annual report to the Administrative Advisor who, upon approval, will send it on to the Western Directors. The Secretary will also record the minutes and preform other duties assigned by the Technical Committee.

To facilitate communications with members, we will continue to support and update the W-187 web site used to develop this proposal. This site is located at http://www.usu.edu/~forestry/w187/. This site will allow members and non-members access to our reports, results and other useful information.

SIGNATURES:

Regional Research Project Number W-187

Interactions Among Bark Beetles, Pathogens, And Conifers in North American Forests

ADMINISTRATIVE ADVISOR

14 Jan 99
DATE

Richard C.
CHAIR, REGIONAL ASSOCIATION OF DIRECTORS

3/24/99
DATE

Administrator, Cooperative State Research, Education and Extension Service

Date

REFERENCES:

Paine, T.D., K.F. Raffa, and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and host conifers. Ann. Rev. Entomol. 42: 179-206.

Schowalter T.D., and G.M. Filip. 1993. Beetle-Pathogen Interactions in Conifer Forests. Academic Press, 252 p.

ATTACHMENTS:

PROJECT LEADERS:

LOCATION	PRINCIPAL OR CO-INVESTIGATORS	COOPERATORS	AREA OF SPECIALIZATION
A. EXPERIMENT STATIONS			
Colorado State	William Jacobi		Forest Pathologist
Iowa State	Tom Harrington		Forest Pathologist/systematist
Oregon State	Darrell Ross		Forest Entomologist
Univ. CA-Riverside	Timothy Paine		Insect/Plant/Microorganism interactions
Univ.CA-Berkeley	David Wood		Forest Entomologist/Physiological Ecology
Univ.CA-Davis	Tom Gordon		Forest Pathologist/Molecular biologist
Univ. Wisconsin	Ken Raffa		Forest Entomologist/microbial interactions
Utah State	Fred Baker		Forest Pathologist/quantitative ecology
B. USDA Forest Service			
Southern Res. Sta.	Kier Klepzig		Forest Entomologist/microbiologist
Rocky Mt. Res. Sta.	Jose Negron		Forest Entomologist
Southern Res. Sta.	John Reeve		Forest Entomologist
C. Other Participants			
Univ.of Montana	Diana Six		Bark beetle/fungal interactions, biological control

RESOURCES

PARTICIPANT	OBJECTIVES				RESOURCES	
	1	2	3	SY %Research%Extension% Teaching	PY	TY
Colorado						
William Jacobi	X			.20	1.00	
Iowa						
Tom Harrington			X	.20		
Oregon						
Darrell Ross	X			.20 15%Research,5%Teaching	.25	
CA-Riverside						
Timothy Paine	X			.35 25%Research,75%Teaching		.60
CA-Berkeley						
David Wood	X			.25		
CA-Davis						
Tom Gordon	X			.20 10%Teaching	.25	.10
Wisconsin						
Ken Raffa	X	X	X	.10 80%Teaching		
Utah						
Fred Baker	X			.10 75%Research,10%Extension 15%Teaching	1.00	
USDA						
Kier Klepzig	X	X	X	1.00 100% Research	1.00	
Jose Negron	X			1.00 100% Research	.40	
John Reeve		X		.50 50% Extension		
OTHER						
Montana						
Diana Six	X		X	.05 67%Research,33%Teaching		

CRITICAL REVIEW:

Work Accomplished Under Original Project W-187
1994-1998

Objective 1: Characterize the role of biotic and abiotic factors in predisposing trees to bark beetle attack and subsequent mortality.

W-187 researchers have been instrumental in contributing to understand the multitude of agents that predispose trees to bark beetle attack. These agents include such things as other insects, fungi, and characteristics of the site where the tree are growing. This discussion will be organized along these lines, however, the interactive nature of these problems makes these categories and the assignment of studies to them difficult. We focus on results, but will report on some of the key studies underway.

Insects Predisposing Trees To Other Insects

The extensive nature of forests often requires sampling to understand forest processes. Data on stand density, stocking, western spruce budworm-caused tree mortality and top kill from 17 stands in New Mexico and Colorado were used to evaluate the precision of various designs for sampling mortality. Sampling efficiency improved as plot size decreased from 0.10-to 0.05-ac designs for all variables and sampling designs. Cluster designs were much more efficient than random sampling designs. On average, 10 pairs of 0.05-ac plots would estimate density, stocking and mortality within a 10% error bound.

Testing hypotheses in the field requires effective and valid techniques for inoculating fungi and initiating insect attacks. Insects use pheromones to initiate a mass attack of an appropriate host. Simply allowing insects to enter a tree does not ensure a realistic inoculation. Tree hammering was studied as a means of simulating beetle attack. By creating many wounds, hammering reduces resin flow which resumes within three days after hammering is terminated. Beetles are more successful in hammered trees, implying they encounter less resin flow.

In the Great Lakes region, red pines were more prone to mortality due to *lps pini* and its principal vectored fungus, *Ophiostoma ips,* if their roots were attacked by *Hylastes porculus, Hylobius radicis,* or *Dendroctonus valens* and its associated fungi *(Leptographium terebrantis, and L. procerum*). Root infected trees were also preferred by *I. pini* in laboratory assays. Drought increased the number of root infected trees killed by *I. pini.* In bioassays, both monoterpenes and phenolics can inhibit both beetles and fungi. Induced monoterpenes reached insecticidal concentrations quickly enough to affect the colonization process. Drought, root infection, and factors that reduce overall carbon stores, such as low light levels, reduce this host response.

In a similar midwestern forest system, severe defoliation of jack pine by the jack pine budworm reduced the rates of preformed resin flow and lesion closure in response to artificial inoculation with *Ophiostoma ips*. Induced monoterpene formation varied with the severity of defoliation, phenology, and time since defoliation. Monoterpene patterns were altered. Annual growth rate and tree survival varied with defoliation, but photosynthesis rates were not affected. Defoliation affected the rates and patterns of tree colonization by *Ips grandicollis and Monochamus spp*. Infestation by other insects may predispose trees to bark beetle attack. Competition with *Monochamus* was an important factor in *Ips* reproduction.

Tree-density management through thinning is one management alternative that could reduce mortality caused by *Dendroctonus* spp. in old-growth ponderosa pine and Douglas-fir and simultaneously retain old-growth characteristics. Tree vigor was assessed two years after treatment by measuring cambial electrical resistance.

Pitch canker caused by *Fusarium circinatum (=F. subglutinans* f. sp. *pini)* has been introduced into California. Infection has resulted in predisposition and death of thousands of trees, particularly Monterey pine, *Pinus radiata*. Pitch canker incidence (proportion of trees with pitch canker symptoms) went from low to high within two to four years. The disease progressed from a few branch tip infections, to many branch tip infections, and ultimately cankers appeared on the main stem. Eighty-seven percent of the trees which died or were removed during the four years of this study had pitch canker. Of all dead or removed trees, 49% had stem cankers, and 66% had more than ten branch tip infections.

The incidence of pitch canker on plots in wild stands has remained around 5% from spring 1996 to spring 1998. Disease incidence on plots in urban areas has increased from 20% to almost 32%, and in heavy urban plots it has increased from 14% to more than 41 % over the same time period. Plots on golf courses had 20% percent severity in spring 1995, and this increased to more than 34% by spring 1998. So far, plots farthest from the coast have less pitch canker than coastal plots.

Monterey pine tips exhibiting several stages of pitch canker infection (green, yellow, and red foliage, and asymptomatic) were reared in the lab, in order to determine how long insects emerging from infected tips carry the pathogen, and how long the pathogen survives in the tip. Most insects emerged from yellow and red tips within approximately 8 weeks, and most of these insects were twig beetles *(Pityophthorus* spp.). Phoresy rates were approximately 10%. Insects feeding ***on*** the tips may also vector the disease. Spittle masses produced by *Aphrophora canadensis* were observed on the green shoots of many trees, and the pitch canker fungus could be isolated from the feeding site of this insect on 55% of branches, and from branch sections adjacent to the feeding site on 29% of branches. In a controlled test, the incidence of pitch canker on branches of potted Monterey pines was dependent on the presence of a spittlebug and on the presence of a spore suspension of the pathogen. A. *canadensis* is a vector of the pitch canker pathogen.

Understanding pitch canker transmission is critical to manage the disease. Seeds from infested areas may carry the pitch canker pathogen and resulting seedlings, if they survive, are often infected. The epidemic of pitch canker has created an abundance of woody refuse. Some of these infested materials are chipped. Insect emergence from chips was reduced by 95-97%, but the fungus could be isolated from 3 month-old chips, and from 2.5 year old tips. The pitch canker fungus was found in 50-75% of the chips from the Monterey Peninsula, and from 15-40% of chips sampled in the Oakland area.

In an attempt to characterize spread of the pitch canker fungus within a stand, a genetically marked strain of *F. circinatum* was released in 1993 on *I. paraconfusus,* but it has not been recovered in subsequent sampling of insects and infected branches. A second marked strain was released on approximately 1800 *Pityophthorus* beetles in a mature Monterey pine stand on the California coast, and analysis is underway.

Host tree resistance will be important to incorporate into regenerated stands of Monterey pine. Lesion lengths produced by Monterey pine in response to artificial inoculations with *F. circinatum* spores increased with increasing spore load up to 250 spores per inoculation. A dose of 125 spores produced significant differences in mean lesion length among trees. Repeated inoculations of trees yielded comparable patterns of resistance among trees over time. Symptoms developing from inoculations with *F. circinatum* werepositively correlated with lesion lengths used as a measure of the tree's response to the pathogen. Of other low elevation conifers, only Pinyon pine and Douglas-fir show some indication of resistance in laboratory studies.

Fungi Predisposing Trees to Insects

In addition to herbivorous insect activity, colonization by fungal pathogens can be important for predisposition of conifers. Predisposing fungi may colonize a range of tissues. For example, Swiss needle cast caused by *Phaeocryptopus gaeumannii* on Douglas-fir slows tree growth and may increase susceptibility to bark beetles. Site quality, stand density, other fungi, and silvicultural practices may either enhance or reduce that predisposition. For example, a study was initiated to determine if thinning can reduce mortality of old growth ponderosa pine and Douglas-fir caused by *Dendroctonus* spp. and still maintain the old-growth stand characteristics. However, several fungi that colonize the main stem and the roots have received the most attention.

Root disease was most severe on drier sites and less severe on dry/low elevation ponderosa pine ecological types. Trees on intermediate sites had more roots than trees on dry/low elevation sites, moist/high elevation sites, or on rocky/lava flows. Trees with more roots may be less prone to stress and therefore less attractive to bark beetles.

Pit-based sampling was used to estimate the proportion of roots with fungal infection symptoms immediately adjacent to spruce trees among the first attacked by spruce beetles in the stand, and at two sample points approximately 100 m from the first sample. Both *I. tomentosus and H. annosum were* found, the latter usually associated with abundant, diseased subalpine fir. More roots with symptoms were found near attacked trees than at the adjacent sites, although symptoms were present in most locations. More roots were encountered at the adjacent sites with less root disease than at the sites where the spruce beetles attacked. Preliminary measurements on two dates in August showed that trees closer to root disease centers and in areas with a higher proportion of symptomatic roots had more negative water potentials on the sunny side of the crown and earlier in the day.

The root pathogen, *Armillaria ostoyae* was associated with declining jack pines in stands infested with *Arceuthobium americanum* in Manitoba. Trees declined as a result of suppression, dwarf mistletoe infection or *Ips* attack.

Thirteen isolates representing 10 clones of *Armillaria ostoyae,* obtained from ponderosa pine, white fir, Douglas-fir, southwestern white pine, blue spruce and aspen, in Northern New Mexico were used to inoculate these same hosts and western larch and lodgepole pine. After 18 months there were no significant differences in mortality among the 8 hosts, or in virulence of the *A. ostoyae* clones, except that the isolate from blue spruce failed to infect any trees. After three growing seasons (30 months), more lodgepole pines were infected than either white fir or Douglas-fir. Ponderosa pine, the dominant species in this region, was similar in susceptibility to infection or mortality to western larch and lodgepole pine.

A study was established to determine if 10-year crop-tree mortality caused by *Armillaria ostoyae and Scolytus ventralis* is significantly affected by silvicultural practices. Tested treatments included 25-acre blocks of clear cutting, commercial thinning, shelterwood, and group selection harvests. Post-harvesting data show that wounding was severe as a result of some treatments.

Considerable interest in cavity nesting wildlife has lead to several projects to use artificial inoculations with decay fungi to create snag habitat. Artificial inoculations may be made by hand, or by shooting bullets carrying wooden dowels colonized by decay fungi from rifles or shotguns. A study was begun in 1994 to determine the successional differences in bark beetle and fungal attack in snags created artificially by topping, girdling, inoculation, or herbicide treatments. Living, dying, and dead trees were artificially inoculated with four species of decay fungi.

Abiotic Factors Predisposing Trees To Insects

Development of *Dendroctonus ponderosae* (mountain pine beetle=MPB) and associated phloem temperatures were intensively monitored in several geographic regions for three generations (three years). The largest proportion of individuals observed in the middle of winter were in the 3rd and 4th larval instars, although all instars were present at that time, as well as during the spring and fall. There were no significant differences in supercooling points among the 4 instars, or between north and south aspects of the bole. Supercooling points and associated phloem temperatures suggest that cold-temperature mortality occurs during the fall and spring. Most individuals sampled could supercool below minimum phloem temperatures during winter. Although MPB populations may have the same capacity to supercool, cold hardiness in any given year and site depends on the temperature regime at that site. A preliminary evaluation of a model of larval development based on field-collected phloem temperatures indicated that the model works well for all but the 3rd instar. The MPB life-systems model is being used to examine the importance of weather on historical MPB outbreaks, and to predict landscape scale representationsof MPB populations.

Environmental factors responsible for shifts in voltinism of *Dendroctonus rufipennis* are currently under investigation. Life-cycles of 1-, 2-, 3- and 4-years have been suggested, although the physiological processes or environmental influences involved remain unclear. A mixture of field and laboratory studies are being combined to more fully understand voltinism and lifestage specific development thresholds of this important insect.

Water-stressed grand fir seedlings were more suitable for insect growth, survival and reproduction. Water stress significantly enhanced infection by *A. ostoyae* and subsequent seedling mortality. However, defoliated seedlings had the lowest *A. ostoyae*-caused mortality in both water regimes.

Five years after fertilization and thinning, intermediated-sized loblolly pine had a significant decrease in resin yield; they apparently put more energy into growth than into secondary metabolism. Codominant trees in thinned stands also showed decreased resin yield. These trees may be more prone to disease. Six years after fertilizing loblolly pines with nitrogen and phosphorus, current diameter growth was not affected, but photosynthesis and height growth were increased. Carbon partitioning to secondary metabolism (including resin synthesis), relative to primary metabolism, in summer was double that in the spring. Beetles introduced into cages were unsuccessful in their attacks on all trees. Following initiation of attacks, which were at low densities compared to commonly observed natural attacks, both control and fertilized trees increased resin production. Oleoresin was synthesized in response to beetle attacks and possibly associated microorganisms.

Codominant trees responded to fertilization and thinning by increasing diameter growth. Conversely, resin flow from bark wounds was less than from codominant trees in unthinned plots regardless of fertilization. Fertilization did not affect diameter growth of codominant trees in unthinned plots. The maximum average resin flow measured among codominant trees was from trees on fertilized, unthinned plots. This response differs from observations in previous years when no apparent differences were found. Intermediate trees on fertilized, thinned plots continue to produce very little resin, but codominant trees in the same plots have greater potential resin yields than codominant trees in unfertilized plots. Only one intermediate tree has died; it was in a fertilized plot. It did not grow in diameter that year, but resin flow had been similar to other intermediate trees until the last two sampling dates, when it dropped to zero.

In a separate study of burning and thinning in loblolly pine stands, more root infesting weevils and beetles carrying *Leptographium* sp. were collected in pitfall traps in thinned plots than in unthinned plots. Fire in longleaf pine stands had no effect on insect number, but root insects numbers were small.

Hazard rating models for the Black Hills National Forest use known points of root disease occurrence to predict the density of *Armillaria* root disease for any point of interest. A trend surface model which incorporates elevation, slope, and site index as independent variables was the best predictor of hazard. Stands with *Armillaria* had more stumps, seedlings and saplings, and less basal area, slightly larger saw timber trees and more basal area in saw timber than stands without the disease. Most trees with *Armillaria* were not in pockets but were scattered in the stands. Incidence of mountain pine beetle was too low to test any relationships with root disease.

During the past 5 years, we have identified several new associations where insects or fungi predispose trees to bark beetles attack. We understand two systems well enough to rate the risk of bark beetle attack in forest stands. Although we have learned much about many other systems operating in our forests, we still lack the understanding needed to prevent bark beetle outbreaks using standard silvicultural techniques such as thinning, fertilization or prescribed burning, especially as we move into the era of ecosystem management.

Objective 2: Characterize interactions among conifer hosts, bark beetles, their natural enemies, and vectored fungi.

W-187 researchers have made considerable progress in understanding the complex relationships between bark beetles, their natural enemies, and vectored fungi. Members Paine, Raffa and Harrington summarized much of this information in a review article published in the Annual Review of Entomology (Paine et al 1998; see attached). What follows here are brief highlights of our work.

The bark beetle genus *Dendroctonus* is the most important genus of tree-killing beetles in North America. We have made significant progress in understanding these insects, but because of the complex interactions in nature, much of our efforts are spent developing techniques to simplify the system so manipulative experiments can be done. Trapping insects, rearing insects, and developing artificial diets are just a few examples of techniques developed by W-187 researchers.

There is considerable interest in understanding the evolutionary and ecological relationships between bark beetles and associated fungi. However, separating the effects of mycangial and non-mycangial fungi on the fitness of the beetles has been difficult. A technique for producing fungus-free beetles and beetles associated with specific mycangial fungi, without using antibiotics and which allowed for maturation feeding, was developed with *D. jeffreyi and D. ponderosae,* two sibling species of bark beetles with maxillary mycangia. Squares of pine bark are cut from uninfested trees, and a small hole is placed half way through the inner bark side of the block thus forming a “pseudo-pupal chamber” (PPC). The blocks are autoclaved and placed into petri dishes with a small layer of water agar. Surface sterilized pupae are placed individually into PPCs to produce fungus-free adults. To produce beetles with a specific fungal associate, the desired fungus is inoculated into the block one week prior to introduction of the pupae. The beetles were left in the PPC for ca. two weeks to allow maturation feeding and acquisition of the fungus in the mycangium. Adults of each species were produced that carried their specific mycangial fungi or that of the sibling species. An attempt to introduce a *Penicillium* species (not normally carried in the mycangium) to mycangia of ten *D. jeffreyi* was unsuccessful. The surface of the beetles was contaminated with the *Penicillium* but the mycangia remained free of fungi, suggesting that the mycangia, are to some degree, selective.

Interactions among fungal associates of bark beetles may be critical to colonization success and beetle fitness. Among fungal associates of *Dendroctonus frontalis* the phoretic, and antagonistic, fungus *Ophiostoma minus* readily outcompetes mycangial fungi *Ceratocystis ranaculosus* and *Entomocorticium* sp. on artificial medium and in loblolly pine logs. *Ophiostoma piliferum*, a fungus used for biocontrol of stain fungi in wood outcompetes all of these *D. frontalis* associated fungi and has potential for use as a biocontrol agent.

Douglas-fir beetle produces broods in dead or fallen western larch, but not in live standing trees. Live western larch is resistant to attacks by the Douglas-fir beetle; the inability of the associated fungi to colonize the host is believed to be involved in host selection by this bark beetle. Two Douglas-fir beetle associated fungi, *Ophiostoma pseudotsugae* and *Leptographium abietinum,* when inoculated into western larch, caused similar phloem necrosis but much less occluded sapwood in larch than in Douglas-fir. Western larch has higher concentrations of the monoterpene, 3-carene. Carene seems to slow attacks, allowing the host to respond, and it is much more toxic to Douglas-fir beetle than alpha-pinene or myrcene. When released with the beetle pheromone, 3-carene reduced trap catches of Douglas fir beetle.

Loblolly pine inoculated with *D. frontalis* associated fungi, and chitosan, a fungal cell wall component and known plant defense elicitor, accumulated large concentrations of ergastic substances.

A synthetic food has been developed and made available to six species of southern pine beetle parasitoid adults. Parasitoid longevity increased when food and water were available compared to water only treatments. Egg resorption decreased and development of new immature eggs increased with parasitoid feeding. The food appears nutritionally complete, is rain-fast, and parasitoids will feed on it in the field. *Thanasimus dubius*, a clerid predator of southern pine beetle, can now be reared on an artificial diet. Researchers hope to produce enough beetles to augment natural populations for biological control. However, results of a long term study of the impact of natural enemies on southern pine beetle suggest that natural enemies had the greatest effect the year after the peak of the outbreak. Natural enemies may contribute to the cycles because they are an agent of delayed density dependence. This same delayed response was observed in populations of *Thanasimus undulatus*, a clerid predator of Douglas-fir beetle.

An extract of trees from southern pine beetle infestations, "Bark OH," *Ips*-infested bolts, plus a fresh-cut bolt and a blank screen were compared for their attractiveness to predators. Two plots of four lines (four traps per line) each (total of 32 traps) were placed for 4 days in logged area with an *lps* brood in the slash. Predator counts were extremely low; only one species was abundant enough to demonstrate significant differences between treatments. The infested bolt treatment attracted more *Palloptera* flies than the other treatments. Two Bracouid parasitoids were attracted only to the attractants, but too few for statistical analysis.

A similar study in August 1996 at the same location compared bark oil from both southern pine beetle and western pine beetle with bolts infested with late instar western pine beetles as a third treatment, and blank traps as controls. Significant numbers of *Medetera and Palloptera* flies were caught, but numbers were not statistically different for any of the treatments. The parasitoid *Dinotiscus* was caught in important, but low, numbers in the infested bolt treatments but in no other treatments.

In another study of the pheromonal/kairomonal interactions among bark beetles and their natural enemies, field assays on behavioral disparities among predators and prey responding to bark beetle pheromones were evaluated. This information provided the basis for a new project on how such disparities can be manipulated for biological control. The major model is *Ips pini, Thanasimus dubius* and two *Platysoma* (Histeridae) species. Additional predators, parasites, and subcortical herbivores (about 15 total) are also being studied. Significant differences were recorded among these groups due to stereochemistry, secondary components, seasonality, and location.

Two Jeffrey pines infested by *lps* species were cut into 30 cm. sections and reared in individual containers. Several species of *lps*, primarily *I. pini*, and several parasitoids, primarily *Roptrocerus* sp. emerged. The number of *Roptrocerus* (841) was similar to the number of *Ips pini* (1027). Predators were absent in at least one year.

Dendroctonus females were found to produce 1 -heptanol; feeding males produced frontalin and exo-brevicomin. Field trials using funnel traps indicated that 1 % or 5% 1 -heptanol in heptane is highly attractive to flying beetles compared to unbaited traps, but the sex ratio of responding beetles was unbalanced (2 males: 1 female).

Fire can predispose trees to bark beetle attacks. After prescribed burning on a study site without root disease, *Dendroctonus valens* and, especially in the tops, *Ips pini* were present. The occurrence and causes of mortality for trees in prescribed burn plots will be determined over a three-year period. Abundance of natural enemies of the bark beetles will be compared in burned and unburned plots to evaluate the impact of prescribed burning on biodiversity and ultimately forest health.

Several years of drought, more than a decade of spruce budworm defoliation, and subsequent attack by *Dendroctonus pseudotsugae* and *Scolytus ventralis,* initiated Douglas-fir mortality. Three years after the onset of mortality, Douglas-fir had 8.1% saprot and grand fir had 1.7%; *Cryptoponus volvatus* was the major cause of decay; and checking was high for both species probably because of the extended drought.

An evaluation of host selection behavior by *Dendroctonus rufipennis,* with particular emphasis on comparisons between outbreak and non-outbreak populations was also initiated in 1997. Populations were collected and assayed from various sources in Alaska, Canadian Yukon, and British Columbia. Separate breeding lines were established. Subsequent molecular comparisons will be conducted. In parallel studies, the heritability of host selection behavior by *lps pini.* is being evaluated. Potential differences in the fungal associates of outbreak versus non-outbreak *D. rufipennis* are being investigated.

Both constitutive and induced responses are required for effective defense of red pines to attack by *lps pini* and their associated *Ophiostoma*. Based on artificial inoculations, *O. pseudotsugae* was more pathogenic to Douglas-fir than was *Leptographium abietinum*. In this system the major predators *are Thanasimus dubius* and two *Platysoma* (Histeridae) species. Significant differences were recorded among these groups due to stereochemistry, secondary components, seasonality, and location. The first of two seasons of field assays exposing logs infested with members of various rearing lines to natural enemy populations have been completed. Chromatographic analyses of beetle variation within populations is continuing. Additional members of the predator and parasite complexes of *I. pini, I. grandicollis,* and *I. perroti* are being identified.

In permanent plots established to monitor the development of pitch canker, 28 of 31 branches which became symptomatic between March and August of 1993 had cone whorls. Cones may be attractive to *Pityophthorus* spp. and *Ernobius punctulatus* which carry propagules of the pitch canker fungus *(Fusarium circinatum).* Infected branches produced large numbers of *P. nitidulus and P. carmeli. Lasconotus pertenuis* (Coleoptera: Scolytidae) has been shown to carry propagules of *F. circinatum.* This beetle is found in *Pityophthorus* galleries and may be a predator of the twig beetles. Approximately 3% of arthropods emerging from cut green branches carried *F. circinatum.* Ninety four percent of these emerged arthropods were *Pityophthorus spp.*; of those contaminated with *F. circinatum, 61* % were *Pityophthorus* spp. and 34% were *Lasconotus spp.* (Colydiidae).

Preliminary findings indicate that *I. paraconfusus* is less likely to oviposit when introduced into Monterey pine logs through holes in which *F. circinatum* was introduced two weeks previously, compared to water treated controls. Tunneling is perpendicular to the grain of the wood in infected logs, until the beetle clears the fungal induced lesion. Where oviposition occurred, the number of eggs laid did not differ between infected and non-infected logs.

Through our collaborative work on objective 2, we are learning to manipulate these complex systems. We can now produce insects which carry no fungi in their mycangium, and we can produce insects which carry fungi that we specify. We can grow their predators on artificial diets. We are learning to manipulate the insects and their natural enemies using pheromones. We still struggle to understand host physiology and how we can modify physiological processes of large trees for manipulative experiments, and ultimately to minimize the impacts of insects and diseases in forest systems.

Objective 3: Characterize the taxonomic diversity and genetic structure of key fungal pathogens and symbiotic fungi associated with insects on North American conifers.

The fir and pine intersterility groups of *Heterobasidion annosum* have unique introns in the mitochondrial large RDNA subunit. The S intersterility group in Europe also has a unique intron, while the P group in Europe lacks an intron in this region. A quick diagnostic procedure, based on the polymerase chain reaction, was developed to distinguish between the S and P strains of *H. annosum*. This new method along with randomly amplified polymorphic DNAs and vegetative compatibility were used to examine the population structure of *H. annosum*. Preliminary data indicates that multiple genotypes are common within centers and within both living and dead individual trees.

Phylogenetic analysis of the DNA sequences of the internal transcribed spacers (ITS) regions and the intergenic spacer region (IGS-1) of the RDNA of isolates from Europe, Japan and western North America indicated that the fir group of *H. annosum* is monophyletic. Isolates of the S and F types from Europe could not be distinguished from each other but formed a clade sister to the Japanese and North American isolates of the fir group. The S type in Europe may be a recently derived form of the fir group, with special adaptations for infection of *Picea abies*. Based on field observations, the S type only occurs in Europe. The western North American group that occurs most commonly on fir and referred to as the S type would best be considered part of the fir group; the S and F types should be distinguished only in Europe. The pine groups in Europe and North America formed distinct clades, and along with *H. insulare,* formed a clade sister to the fir group. A new technique for examining polymorphisms in the mitochondrial genome of fungi was demonstrated with *H. annosum.*

Many clones of *Armillaria ostoyae* attack ponderosa pine, aspen and spruce in the Black Hills. One clone in the survey covered approximately 1,280 ft. along a transect. Polymerase chain reaction amplification of the intergenic spacer region of the RDNA and restriction digests of the amplicon revealed profiles which served to distinguish species of *Armillaria* in Europe and North America. This procedure greatly shortens the time required for species identification from approximately two months to less than eight hours.

The population structure of *Fusarium circinatum,* cause of pitch canker, in California was characterized based on vegetative compatibility. Eight vegetative compatibility groups (VCGS) have been identified in the California population, with a single VCG predominating in the central coastal region of California where pitch canker is most severe. The data suggest that the expansion of pitch canker in this region is attributable to local, tree to tree, spread of the disease. In contrast, a VCG previously identified only in a Southern California Christmas tree nursery, was recently found in Northern California locations, suggesting long distance disease spread via movement of infected trees. Based on both randomly amplified polymorphic DNA and anonymous genomic clones, isolates associated with the different VCGs are identical. Polymorphisms in the intergenic spacer region distinguish one VCG (C3) from all the others.

Based on an examination of enzymes associated with l6 different loci (7 of which were polymorphic), allele frequencies differed significantly between three montane California populations of *Dendroctonus jeffreyi*. The Sierra Nevada populations were not clearly differentiated based on allele frequencies but the southern California populations were clearly distinct. This latter finding is presumed to reflect the geographic isolation of the southern California population. In contrast, in the mycangial fungus associated with *D. jeffreyi,* only one of 12 loci proved to be polymorphic among isolates obtained over a broad geographic area. In another study, of twenty-one loci assayed, seven were polymorphic in at least one population. Average heterozygosity across all populations and loci was 4%. Genetic identity ranged from 0.998 to 0.931. Two southern populations from the San Bernardino Mountains were the most differentiated. At three loci, alleles were present in the southern populations that were absent from northern populations. The southern populations also lacked an allele at one locus that was present in all northern populations. Geographic isolation is apparently responsible for allowing the divergence of these populations. Dendrograms estimating the relationships among the ten populations were developed using three methods: UPGMA, restricted maximum likelihood, and parsimony. The restricted maximum likelihood tree best reflected geographic relationships among the beetle populations. Significant departures from Hardy-Weinberg expectations for random mating were found in all ten populations surveyed. Inbreeding coefficient values indicated that 43% of the reduction in heterozygosity within populations was due to non-random mating or inbreeding. Inbreeding in these beetles may be closely tied to population size and dispersal behavior.

The two major mycangial fungi of *Dendroctonus brevicomis* are similar to but distinct from those of *D. frontalis*. The basidiomycete symbionts of the two beetles are closely related but unnamed species and may be related to *Peniophora* species based on DNA sequence comparisons. These fungi may be mutualistic with bark beetles by providing nutritional benefit. *Entomocorticium dendrocton,* other associates of *D. ponderosa and D. jeffreyi,* and the mycangial fungus of *Pityoborus comatus* are in this group of basidiomycetes. *Phlebiopsis gigantea* was identified through DNA sequence analysis as the arthroconidial basidiomycete recorded as an associate of *Dendroctonus ponderosae.* This same fungus was isolated from the mycangium of *D. approximatus,* which was surprising because the basidiomycetes associated with the mycangium of the closely related *D. brevicomis and D. frontalis are Entomocorticium* species (sp. B and sp. A, respectively).

The mycangial fungi associated with the sibling species *D. jeffreyi and D. ponderosae* were apparently derived from a common ancestor but not to a degree resolvable by isozyme electrophoresis. The *Ceratocystiopsis* species associated with D. *brevicomis* is more closely related to *C. collifera* than to the *D. frontalis* associate C. *ranaculosus,* and was described as the new species *C. brevicomi.* Two new species of *Ceratocystis* were also described; *C. rufipenni,* an associate *of D. rufipennis* and pathogenic to inoculated spruce trees, and *C. douglasii (C coerulesceits f. douglasii)* which causes a stain of Douglas-fir but is not known to be associated with bark beetles.

Using morphology, isozyme phenotypes, and growth rates at different temperatures, all isolates from mycangia of more than 900 *D. jeffreyi* collected from a large portion of its geographic range were *Ophiostoma clavigerum; O. montium* was never isolated from *D. jeffreyi* mycangia. Attempts to produce the teleomorph of *O. clavigerum* were unsuccessful. Fungi from mycangia of *D. adjunctus* were isolated and compared with *Leptographium pyrinum* and *O. adjuncti,* two species of fungi known to be present in trees colonized by *D. adjunctus.* Fungi isolated from *D. adjunctus* mycangia were morphologically and genetically identical to *L. pyrinum.*

Genetic variation within and among ten populations of *0. clavigerum* associated with the mycangia of *D. jeffreyi* was assessed using isozymes. Only two of nineteen loci assayed were polymorphic. Gene diversity was uniformly low across all populations with an average of 1.4%. The three most southerly populations, two from the San Bernardino Mountains in southern California and one from the southern tip of the Sierra Nevada range, were the most differentiated. However, all populations exhibited a very high level of identity (all pairwise comparisons exceeding 0.990). Several factors alone, or in concert, could result in the low genetic diversity and high genetic identity observed in these populations: a lack or rarity of sexual recombination, stabilizing selection, a history involving genetic bottlenecks, and selection for a mutualistic association with the host beetle. Dendrograms were produced using three methods: UPGMA, restricted maximum likelihood, and parsimony. The restricted maximum likelihood tree best reflected geographic relationships among the fungal populations.

The fungal genus *Ambrosiella* was polyphyletic and consisted of two distinct groups: one associated with scolytid beetles and closely related to *Ceratocystis*, the other showed a close relationship to *Ophiostoma*. *The Ophiostoma* group displays cycloheximide resistance. These species are associated with scolytid and platypoid beetles. *Pyxidiophora*, a genus of many species which are saprobic in bark beetle galleries, has affinities with the Laboulbeniales. *Symbiotaphrina*, a yeast-like inhabitant of the gut of Anobiids is not a relative of *Taphrina*, as once thought, but rather is part of an early rapid divergence of discomycete and loculoascomycete fungi.

W-187 has served as a major clearinghouse for research on the taxonomy and genetic structure of insect associated fungi. We have been instrumental in advancing knowledge in two areas: *Heterobasidion annosum*, the most important root disease affecting conifers worldwide, and in the major bark beetle vectored fungi, especially the Ophoistomatoid fungi. Our continued efforts will lead to an understanding of the evolution of both fungi and bark beetles.

USEFULNESS OF THE WORK

W-187 has made many important contributions that will help forest managers be better stewards of the forests. Establishing correlations between site characteristics and severity of root disease will help managers anticipate and therefore more effectively manage root disease and the resultant beetle attack. In some cases, data acquired will be used to develop models that can predict the development of an insect, e.g. the mountain pine beetle, or the progress of a disease. Such models will be helpful in understanding triggering events that precipitate shifts in population status from the endemic to epidemic phase. Understanding the effects of management practices on tree physiology will help plantation managers avoid predisposing their trees to disease and insect problems. The demonstration of a negative relationship between vigorous growth and resin synthesis is contrary to the widely held view that vigor is positively correlated with resistance to bark beetles. This finding will allow plantations managers to make better informed decisions concerning fertilization practices. The development of better methods for the establishment of wildlife trees will assist forest managers in achieving the objective of maximizing biodiversity. All of these finding are contributing to a better understanding of factors predisposing trees to bark beetle attack.

Our efforts in studying the interactions between the players in this complex system have already provided better research tools for trapping insects and identifying fungal strains. For example, the time required to identify species of *Armillaria* has been reduced from 6-8 weeks to 6 hours. These tools will enable researchers to investigate questions regarding contributions of beetles or symbiotic fungi to the fitness of the other organism and how that relates to the host trees.

These efforts have also had many more practical results. Monitoring the movement of a marked strain of *F. circinatum* will elucidate the pattern of spread of pitch canker and allow for better predictions of how the disease will spread. This information will contribute to management of this disease in California, and prevention of its spread to other areas. Successful development of synthetic diets for parasitoids could result in a biological control program for southern pine beetle. Further, understanding the response of target insects and their natural enemies to pheromones will allow us to use this tool to manage the insect population, either directly, or by increasing the response of predators and parasites to the infestation. The ability to manipulate fungal associates may allow management of insect populations by interfering with the fungi they need to successfully develop broods within their host.

Our main focus has been, and will continue to be, on the interactions among elements of the biotic environment including bark beetles, host conifers, associated fungi, natural enemies, pathogenic fungi, herbivores and the abiotic environment that makes a forest ecosystem. Understanding how those elements interact is critical to develop management schemes to ensure that the forest is sustainable and productive. While applicable to production forests, preserved old-growth, and plantations, the results have been particularly valuable recently in developing management plans to reduce risk of bark beetle-caused mortality in high value urban forests.

We have studied the phylogenetic evolutionary relationships among symbiotic fungi of a range of primary bark beetles, and have quantified the potential impact of successive evolutionary bottlenecks on the population genetics of these ecologically linked associations. Yet, we understand only a few of the more intensively studied systems, primarily involving species of *Dendroctonus*. Bark beetles are associated with a complex mycoflora, and many of these fungi play a critical role in beetle biology. We now suspect that *Ceratocystis* species, rather than *Ophiostoma or Leptographium,* are the most important component of the microflora that is pathogenic to conifers. Also, basidiomycetes may play an important nutritional role for some of the most important bark beetle species, but these basidiomycetes have been very poorly known.

Many of the techniques and results of W-187 studies are contributing to an understanding of the risk of importation of new insects, fungi, and genotypes of existing insects and fungi on logs. W-187 members are actively involved in the issue of timber import, and are creating an awareness of this issue. Continued development of this knowledge will be extremely helpful when new pests become established.

Publications

Baker, F.A. and S. Durham. 1993. Relationship of annosus root disease to ponderosa pine ecological and site characteristics. USDA Forest Service, Forest Pest Management, Report on Participating Agreement PA -5-91-20-201.

Baker, F.A. 1997. Getting to the root of Forest Disease. UT Science58:10-11.

Baker, F.A. 1998. Pioneering spruce beetles attack Engelmann spruce in stand areas with more root disease. 1998 American Phytopathological Society, Las Vegas, NV. Abstract published in Phytopathology 88(9, supplement) S5.

Barry, D.L., G.M. Filip, SA. Willits, and CA. Parks. 1996. Lumber recovery and deterioration of beetlekilled Douglas-fir and grand fir in the Blue Mountains of eastern Oregon. USDA For. Serv. Gen. Tech. Rept. PNW-GTR-376, Portland, OR. 24p.

Bentz, B.J. 1995. Ecological adaptations of the mountain pine beetle to cold temperatures. In: Proceedings: Joint IUFRO Conference: Behavior, Population Dynamics and Control of Forest Insects, Maui, HI (In Press).

Berisford, C.W., D.L. Dahisten and F.D. Morgan. 1995. Classical biological control of *lps grandicollis* (Eichoff) (Coleoptera: Scolytidae) in Australia: Impact of a single species of parasitioid. IUFRO Proceedings. Maui, HI (in Press).

Berisford, D. W., Dahlsten, D. L. and F. D. Morgan. 1995. Classical biological control of *Ips grandicolis* (Eichoff) (Coleoptera:Scolytidae) in Australia: Impact of a single species of parasitoid. In: Hain, F. P., T. L. Payne, W. F. Ravlin, Salom, S. M. & K. F. Raffa (eds.), "Behavior, Population Dynamics and Control of Forest Insects." Proc. International Union of Forestry Research Organizations.

Blackwell, M., J.W. Spatafora, D. Malloch and J.W. Taylor. 1993. Consideration of higher taxonomic relationships involving *Pyxidiophora.* p. 105-108. In: *Ceratocystis and Ophiostoma:* Taxonomy, Ecology and Pathology. Eds., M.J. Wingfield, K.A. Siefert and J.F. Webber. APS, St. Paul.

Blackwell, M., and J.W. Spatafora. 1994. Molecular data sets and broad taxon sampling in detecting morphological convergence. In: First International Workshop on Ascomycete Systematics. Ed., Hawksworth, D.L. Plenum Press, New York.

Espelie, K. D., C. W. Berisford and Dahlsten, D. L. 1995. Use of cuticular hydrocarbons in bark beetle parasitoid taxonomy: a study of *Roptocerus xylophagorum* (Ratzeburg) (Hymenoptera:Torymidae) from the United States, Europe and Australia. Comparative Biochemistry and Physiology, Section B.

Dahlsten, D.L. and C.W. Berisford. 1995. Diversity of bark beetle natural enemies. In: biology and implementation of bark beetle natural enemies, IUFRO Proceedings. Maui, HI (In Press).

Dallara, P. L. 1993. Interrelationships among twig beetles (Scolytidae: *Pityophthorus* spp.), pitch canker disease, and conifers in central coastal California. In: Proceedings of the 42nd. Annual Meeting of the California Forest Pest Council. Eds., D. Adams, J. Rios and A. J. Storer. p. 44-45.

Dallara, P. L., A. J. Storer, T. R. Gordon, and D. L. Wood. 1995. Current status of pitch canker disease in California. Tree Note 20. 4 pages.

DeScenzo, R. A., and T. C. Harrington. 1994. Use of (CAT)5 as a DNA fingerprinting probe for fungi. Phytopathology 84:534-540.

Filip, G. M. 1994. Forest Health decline in central Oregon: A 13-year case study. Northwest Science 68:289-296.

Filip, G. M., and D. J. Goheen. 1995. Precommercial thinning in *Pseudotsuga, Tsuga, and Abies* stands affected by Armillaria root disease: 10-year results. Can. J. For. Res. 25:817-823.

Filip, G. M., A. Kanaskie, and A. Campbell III. 1995. Forest disease ecology and management in Oregon. Extension Manual 9, Oregon State University Extension Service, Corvallis. 60 p.

Gordon, T. R., A. H. McCain, M. E. Schultz, C. S. Koehler, J. W. Fox, and D. L. Wood. 1994. Epidemiology and control of pitch canker in pines in California. In: Innovations in Landscape Pest Management. J. White (ed.). Division of Agriculture and Natural Resources, University of California. pp. 15-17.

Gordon, T. R. 1994. Pitch canker in Monterey pines. In: Proceedings of the 43rd Annual meeting of the California Forest Pest Council., D. H. Adams, J. E. Rios and A. J. Storer, eds.

Gordon, T. R., A. J. Storer and D. Okamoto. 1996. The population structure of the pitch canker pathogen, *Fusarium subglutinans* f. sp. *pini,* in California. Mycological Research (in press).

Gordon, T.R. Wikler, K.R., Clark, S.L., Okamoto, D., Storer, A.J. and Bonello, P. 1998. Resistance to pitch canker disease, caused by Fusarium subglutinans f. sp. pini, in Monterey pine (Pinus radiata). Plant Pathology 47:706-711.

Gordon, T.R., Storer, A.J., Okamoto and Wood, D.L. 1998 Susceptibility of five landscape pines to pitch canker, caused by Fusarium subglutinans f. sp. pini. HortScience 33:868-871.

Harrington, T. C., D. M. McNew, J. P. Steimel, and M. J. Wingfield. 1994. Variation in isozymes and morphology of species of *Ceratocystis* sensu stricto on conifers. Proc. 5th Intern. Mycological Congress, Vancouver, Canada. p. 85. (abstract).

Harrington, T. C. 1995. Clonal development of root-rotting basidiomycetes in forest stands. South African J. Plant Pathol. 9 1:1. (abstract).

Harrington, T. C., and B. D. Wingfield. 1995. A PCR-based identification method for species of *Annillaria*. Mycologia 87:280-288.

Harrington, T. C., and D. M. McNew. 1995. Mating type switching and self-fertility in *Ceratocystis*. Inoculum 46:18 (Mycological Society of America) (abstract).

Harrington, T. C., J. Steimel, M. J. Wingfield, and G. A. Kile. 1996. Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. Mycologia (in press).

Harrington, T. C., and M. J. Wingfield. 1996. Diseases and the ecology of indigenous and exotic pines. In: *Ecology and Biogeography of* Pinus, D. Richardson, (ed.). Cambridge University Press (in press).

Harrington, T. C., and M. **J.** Wingfield. 1996. Diseases and the ecology of indigenous and exotic pines. in: D. Richardson, cd. *Ecology and Biogeographlty of* Pinus. Cambridge University Press (in press).

Harrington, T. C., **J.** Steimel, M. **J.** Wingfield, and G. A. Kile. 1996. Isozyme variation and species delimitation in the *Ceralocystis coerulescens* complex. Mycologia 88:104-113.

Hebertson, E.G. 1995. Associations of Armiflaria root disease in jack pine with Arceuthobium americanum, M.S. Thcsis, Utah State University, 107 pp.

Hobson, K. R., J. R. Parmeter, Jr., and D. L. Wood. 1994. The role of fungi vectored by *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae) in occlusion of ponderosa pine xylem. Can. Ent. 126:277-282.

Hoffman, G.D., D.W.A. Hunt, S.M. Salom, & K.F. Raffa. 1997. Reproductive readiness and niche differences affect conifer root weevil responses to simulated host odors. Environ. Entomol. 26:91-100.

Hoover, K., D. L. Wood, **J.** W. Fox and W. E. Bros. 1995. Quantitative and seasonal association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini, with Conophthorus radiatae* (Coleoptera: Scolytidae) and *Emobius punctulatus* (Coleoptera: Anobiidae) which infest *Pinus radiata*. Canadian Entomologist 127:79-91.

Hsiau, P. T. W., and T. C. Harrington. 1994. Comparison of mycangial fungi from *Dendroctonus brevicomis and D. frontalis* (Coleoptera: Scolytidae). Proc. 5th Intern. Mycological Congress, Vancouver, Canada. p. 95. (abstract).

Hsiau, P. T. W., and T. C. Harrington. 1995. Basidiomycetes associated with bark and ambrosia beetles (Coleoptera: Scolytidae). Inoculum 46:20. (Mycological Society of America) (abstract).

Kallas, M. A. 1997. Armillaria root disease in the Black Hills of South Dakota. M. S. Thesis, Colorado State University. 172 pp.

Kile, G. A., T. C. Harrington, Z. Q. Yuan, M. J. Dudzinski, and K. M. Old. 1996. *Ceralocystis eucalypti* sp. nov., a vascular stain fungus from eucalypts in Australia. Mycol. Res. 100:571-579.

Kile, G. A., and T. C. Harrington. 1994. A new *Ceratocystis* species from Eucalyptus and its relationship to *C. virescens and Chalara australis*. Proc. 5th Intern. Mycological Congress, Vancouver, Canada. p. 109. (abstract).

Kirkendall, L.R., D.S. Kent, & K.F. Raffa. 1996. Interactions among males, females and offspring in bark and ambrosia beetles: The significance of living in tunnels for the evolution of social behavior. Pp. 181-215. In, J.C. Choe & BJ. Crespi, (eds.), The Evolution of Social Behavior in Insects and Arachnids, Cambridge Univ. Press, Cambridge.

Klepzig, K.D. 1998. Competition between a biological control fungus, *Ophiostoma piliferum*, and symbionts of the southern pine beetle. Mycologia. 90:000-000.

Klepzig, K.D., Smalley, E.B., & K.F. Raffa. 1996. Combined chemical defenses against insects and fungi associated with a forest decline disease. J. Chem. Ecol. 22: 1367-1388.

Klepzig, K.D., E.B. Smalley, & K.F. Raffa. 1996. Interactions of ecologically similar saprogenic fungi and abiotically stressed conifers. Forest Ecol. & Management. 86: 163-169.

Klepzig, K.D., and R.T. Wilkens. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. Appl. Env. Microbiol. 63:621-627.

Klepzig, K.D., E.L. Kruger, E.B. Smalley, & K.F. Raffa. 1995. Effects of biotic and abiotic stress on the induced accumulation of terpenes and phenolics in red pines inoculated with a bark beetle vectored fungus. J. Chem. Ecol. 21:601-626.

Klepzig, K.D., K.F. Raffa, & E.B. Smalley. 1995. *Dendroctonus valens and Hylastes porculus* (Coleoptera: Scolytidae) vectors of pathogenic fungi (Ophiostomatales) associated with Red Pine Decline Disease. The Great Lakes Entomol. 28:81-87.

Krokene, P., K.F. Raffa, & L.R. Kirkendall, Tree - killing in conifer bark beetles: the importance of phytopathogenic fungi. Subm. To the Ecol. Entomol.

Logan, J.A., P.V. Bolstad, B.J. Bentz and D.L. Perlkins. 1995. Assessing the effects of changing climate on mountain pine beetle dynamics. In: Proceedings: Joint IUFRO Conference: Behavior, Population Dynamics and Control of Forest Insects, Maui, HI (in Press).

Lorio, P.L., Jr. 1993. Environmental stress and whole-tree physiology. In: Beetlepathogen interactions in conifer forests. Eds., T.D. Schowalter and G.M. Filip. Academic Press, London.

Lorio, P. L., Jr. 1994. The relationship of oleoresin exudation pressure (or lack thereof) to flow from wounds. **J.** Sustainable Forestry 1(4):81-93.

Lorio, P. L., Jr., F. W. Stephen, and T. D. Paine. 1995. Environment and ontogeny modify loblolly pine response to induced acute water deficits and bark beetle attack. For. Ecol. Manag. 73:97-110.

Lorio, P.L., Jr. 1996. Approaches to studying environmental effects on resistance to Pinus taeda L. to Dendroctonus frontalis Zimmermann, In Dynamics of Forest Herbivory: Quest for Pattern and Principle (Mattson, WJ., Niemela, P., and Rousi, M., eds.). USDA For. Gen. Tech. Rep. NC-183, N.C. For. Exp. Sta., St. Paul, MN 55108

Lundquist, J.E. 1995. Pest Interactions and canopy gaps in ponderosa pine in the Black Hills, South Dakota. Forest Ecology and Management (In Press).

Lundquist, J.E. 1995. Disturbance profile - a measure of small-scale disturbance patterns in ponderosa pine stands. Forest Ecology and Management (In Press).

Lundquist, J.E. 1995. Characterizing disturbance in managed ponderosa pine stands in the Black Hills. Forest Ecology and Management (in Press).

McNulty, S.G., Lorio, P.L., Jr., Ayres, M.P. and Reeve, J.D. (In Press) Predictions of southern pine beetle populations under historic and projected climate using a forest ecosystem model. In The Productivity and Sustainability of Southern Forest Ecosystems in a Changing Environment (R. Mickler, ed.), Springer-Verlag

McPheron, L. J., S. J. Seybold, A. J. Storer, D. L. Wood, T. Ohtusuka and I. Kubo. 1997. Effects of enantiomeric blend of verbenone on the response of *lps paraconfu.sus* Lanier to naturally produced aggregation pheromone in the laboratory. *J. Chem. Ecol.* 23, in press.

Miller, D.R., K.E. Gibson, K.F. Raffa, S.J. Seybold, S.A. Teale & D.L. Wood. 1997. Geographic variation in the response of the pine engraver, Ips pini (Say) (Coleoptera: Scolytidae) and associated species to the pheromone, lanierone. J. Chem. Ecol. 23: 2013-2031.

Omdal, D.W. 1994. *Armillaria ostoyae:* A pathological perspective from the Southern Rocky Mountains. Ph.D. Dissertation. Colorado State University. 154 p.

Omdal, D.W., C.G. Shaw, W.R. Jacobi and T.C. Wager. 1993. Pathogenicity and virulence of *Armillaria ostoyae* on eight forest tree species. Phytopathology 83:1365 (abstr.)

Omdal, D. W., C. G. Shaw, III, W. R. Jacobi, and T. C. Wagner. 1995. Variation in pathogenicity and virulence of isolates of *Annillaria ostoyae* on eight tree species. 79:939-944.

Paine, T. D., and C. C. Hanlon. 1994. Influence of oleoresin constituents from *Pinus ponderosa and Pinus jeffreyi* on the growth of mycangial fungi from *Dendroctonus ponderosae and Dendroctonusjeffreyi*. **J.** Chem. Ecol. 20:2551-2563.

Paine, T.D., K.F. Raffa, & T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and host conifers. Ann. Rev. Entomol. 42: 179206.

Raffa, K.F., Krause, S.C., & P. Reich. Long-term influence of defoliation on Pinus resinosa suitability to diverse insect herbivore guilds. Ecology. In press.

Raffa, K.F. and K.D. Klepzig. 1994. Effects of root inhabiting insect-fungal complexes on aspects of host resistance to bark beetles. In: W. Mattson and P. Niemela. Mechanisms of Woody Plant Defenses against Herbivores. Proc. Int. Union of Forestry Research Organizations.

Raffa, K.F. 1994. Differential responses among natural enemies and prey to bark beetle pheromones: Implications of chemical, temporal and spatial disparities to evolutionary theory and pest management. In F. Hain, T. Payne, H. Saaraneema and K.F. Raffa. Behavior, Population Dynamics and Control and its Rationale. Proc. Int. union of Forestry Research Organizations.

Raffa, K. F. 1995. Differential responses among natural enemies and prey to bark beetle pheromones: Implications of chemical, temporal, and spatial disparities to evolutionary theory and pest management. In: Hain, F. P., T. L. Payne, W. F. Ravlin, Salom, S. M. & K. F. Raffa (eds.), "Behavior, Population Dynamics and Control of Forest Insects." Proc. International Union of Forestry Research Organizations.

Raffa, K. F. 1995. Population ecology and behavior of root-damaging Hylobius in North America. In: Hain, F. P., T. L. Payne, W. F. Ravlin, Salom, S. M. & K. F. Raffa (eds.), "Behavior, Population Dynamics and Control of Forest Insects." Proc. International Union of Forestry Research Organizations.

Raffa, K.F. 1995. Bark beetle, fungi, trees, and humans: Four perspectives, four agendas. Pp. 7-9, In Christiansen (ed.), Bark Beetle, Blue-Stain Fungi, and Conifer Defense Systems Nr. 6-95.

Raffa, K.F. 1996. Population ecology and behavior of root-damaging Hylobius in North America. Proc. International Union of Forestry Research Organizations.

Raffa, K. F. & D. L. Dahlsten. 1995. Differential responses among natural enemies and prey to bark beetle pheromones. Oecologia. 102:17-23.

Raffa, K. F. & E. B. Smalley. 1995. Interaction of pre-attack and induced monoterpene concentrations in conifer defense against bark beetle-microbial complexes. Oecologia. 102:285295.

Raffa, K.F. & D.L. Dahlsten. 1995. Differential responses among natural enemies and prcy to bark beetle pheromones. Occologia. 102:17-23.

Raffa, K.F. & K.D. Klepzig. 1996. Effects of root inhabiting insect-fungal complexes on aspects of host resistance to bark beetles. In, Mattson, W., Niemela, P., & Rousi, M. (eds.), Dynamics of Herbivory: Quest for Pattern and Principle. Proc. International Union of Forestry Research Organizations. USDA FS Gen. Tech. Rept. NC-183.

Raffa, K.F. Semiochemical disparities among bark beetles & natural enemies responding to bark beetle pheromones. In: Hayes, J. & K. Raffa (eds.), Proceedings Bark Beetle Genetics Workshop. USDA FS Gen. Tech. Rept. In press.

Rappaport, N. G. and D. L. Wood. 1994. *Pityophthorus orarius* Bright (Coleoptera: Scolytidae) in a Northern California Douglas fir seed orchard: Effect of clone, tree vigor, and cone crop on rate of attack. Can. Ent. 1111-1118.

Ravlin, Salom, S. M. & K. F. Raffa (eds.), "Behavior, Population Dynamics and Control of Forest Insects." Proc. International Union of Forestry Research Organizations.

Reeve, J. D., M. P. Ayres, and P. L. Lorio, Jr. 1995. Host suitability, predation, and bark beetle population dynamics. pp. 339-357. In: *Population Dynamics: New Approaches and Synthesis,* N. Cappuccino and P.W. Price, (eds.). Academic Press, Inc, San Diego, CA, 429 p.

Rieske, L.K., and K.F. Raffa. Use of baited pitfall traps, and evaluation of dispensing methods, for root weevils in newly established pine plantations in Wisconsin. J. Econ. Entomol. In press.

Ross, D. W., and H. Solheim. 1995. Pathogenicity of Douglas-fir beetle associated fungi, *Ophiostoma pseudotsugae and Leptographium abietinum,* to Douglas-fir. In: Bark Beetles, Bluestain Fungi, and Conifer Defense Systems, E. Christiansen (ed.). Proc. from a symp. held at the Norwegian Forest Research Institute (NISK) in ks, Norway, 31 July - 2 August 1995. NISK, As, Norway. Nr. 6-95. p. 17.

Ross, D.W. and H. Solheim. 1995. Douglas-fir and western larch defensive reaction to *Leptographium abietinum and Ophiostoma pseudotsugae.* In: Proceedings IUFRO working party S2.05-06, Mechanisms of woody plant defense against herbivores, Maui, HI (In Press).

Ross, D. W., and H. Solheim. 1996. Douglas-fir and western larch defensive reactions to *Leptographium abietinum and Ophiostoma pseudotsugae.* In Dynamics of Forest Herbivory: Quest for Pattern and Principle. W. J. Mattson, P. Niemlam, and M. Rousi (eds.). USDA Forest Service General Technical Report NC- 1 83. pp. 224-227.

Ross, D. W., and H. Solheim. 1997. Pathogenicity of Douglas-fir beetle associated *fungi, Ophiostoma pseudotsugae and Leprographiwn abietinum* to Douglas-fir. Can. J. For. Res. 27:39-43.

Six, D. L., and T. D. Paine. 1997 *Ophiostoma clavirerum* is the mycangial fungus of the Jeffrey pine beetle, Dendroctonus jeffreyi. Mycologia 89:858-866.

Six, D. L., and T. D. Paine. A phylogenetic comparison of ascomycete mycangial fungi and *Dendroctonus* bark beetles (Coleoptera: Scolytidae). Ann. Entomol. Soc. Amer. (In Press)

Storer, A. J., T. R. Gordon, P. L. Dallara and D. L. Wood. 1994. Pitch canker kills pines, spreads to new species and regions. California Agriculture, 48: 9-13.

Storer, A. J., T. R. Gordon, D. L. Wood and P. L. Dallara. 1995. Entomological and pathological aspects of pitch canker disease in California. In: Proceedings of the International Union of Forest Research Organizations, Maui, Hawaii (In Press).

Storer, A. J., T. R. Gordon, D. L. Wood and P. Bonello. 1997. Current and future impacts of pitch canker disease of pines. J. For. 95(12), in press.

Storer, A. J., T. R. Gordon, D. L. Wood and P. L. Dallara. 1995. Entomological and pathological aspects of pitch canker disease in California. In: Hain, F. P. , T. L. Payne, W. F. Sung, Jae-Mo, Bum-Shig Jung, Keun-Joo Yang, Hyun-Kyung Lee, and T. C. Harrington. 1995. Production of *Gastrodia elata* tuber using *Armillaria* spp. Korean J. Mycol. 23:61-70.

Storer, A.J., Gordon, T.R. and Clark, S.L. 1998. Association of the pitch canker fungus, Fusarium subglutinans f. sp. pini, with Monterey pine seeds and seedlings in California. Plant Pathology 47:706-711.

Storer, A.J., Wood, D.L., Wikler, K. and Gordon, T.R. 1998. Association of a native spittlebug (Homoptera: Cercopidae) on Monterey pine, and an introduced tree pathogen which causes pitch canker disease. Canadian Entomologist: In Press.

van der Westhuizen, K., M. J. Wingfield, D. R. Bergdahl, and T. C. Harrington. 1995. A new species of *Ophiostoma* from larch in North America. South African J. Plant Pathol. 91:xvii.

Wallin, K.F., & K.F. Raffa. Effect of Choristoneura pinus pinus (Lepidoptera: Tortricidae) feeding on components of Pinus banksiana resistance to subcorfical insect - fungal complexes. Ecology. In press

Wallin, K.F. & K.F. Raffa. 1998. Association of within-tree and within-needle variation of water, nutrient and monoterpene concentrations with canopy level and jack pine budworm feeding patterns. Can. J. For. Res. 28: 228-233.

Wallin, K.F., and K.F. Raffa. Altered constitutive and inducible phloem monoterpenes following natural defoliation of jack pine: Implications to host mediated inter-guild interactions and plant defense theories. J. Chem. Ecol. In press.

Warren, C. E., D. L. Wood, S. J. Seybold, A. J. Storer and W. E. Bros. 1996. The olfactory response of *Ips plastographus maritimus* Lanier (Coleoptera: Scolytidae) to insect and host associated volatiles in the laboratory. *J. Chem. Ecol.* 22: 22992316.

Wilkens, R.T., Ayres, M.P., Lorio, P.L., Jr. and Hodges, J.D. (In Press) Environmental effects on pine tree carbon budgets and resistance to bark beetles. In The Productivity and Sustainability of Southern Forest Ecosystems in a Changing Environment (R. Mickler, ed.), Springer-Verlag

Wingfield, M. J., T. C. Harrington, and P. W. Crous. 1994. Three new *Leptographium* species associated with conifer roots in the United States. Can. J. Bot. 72:227-238.

Bryant, J., & K. F. Raffa. 1995. Chemical Antiherbivore Defense. Pp.365-381, in B.L. Gartner (ed.), Stems and Trunks in Plant Form and Function. Academic Press.

Wingfield, B. D., T. C. Harrington, and J. Steimel. 1996. A simple method for detection of mitochondrial DNA polymorphisms. Fungal Genetics Newsletter 43:56-60 .(http://www.kumc.edu/research/fgsc/fgn43/wingfield.html).

Paine, T.D., K.F. Raffa, & T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and host conifers. Ann. Rev. Entomol. 42: 179-206.

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