AN ABSTRACT OF THE THESIS OF

Carlos M. Garcia for the degree of Master of Science in Forest Products presented on September 13 , 1996. Title: Fungal Associates in the Golden Metallic Beetle, ***Buprestis aurulenta L.***

Abstract approved: Signature redacted for privacy.

Jeffrey J. Morrell

This study determined the role of ***Buprestis aurulenta*** L. as a potential vector of basidiomycetes and other microfungi in lumberyards. Fungal isolations from adult beetles collected from the log decks were made by planting external parts (head, thorax, legs and elytra), by planting the gut, by allowing the insect to crawl or by streaking the washings from the body of beetles on plated culture media. Decay tests, growth patterns and reactions on specific media were performed on five basidiomycetes isolated from the beetles. The external surfaces of the body of the beetle were examined under the scanning electron microscope to determine the structures implicated in the transport of fungi.

A total of 863 fungal isolates were obtained from the external parts and gut of 80 beetles. Basidiomycetes were

less frequently isolated (2.8%) while fungi imperfecti constituted 92.7% of the total isolates. Yeasts represented 4.5% of all isolates.

Five basidiomycetous fungi were isolated from the body parts of the beetle namely ***Basidio #1***, ***Polyporus #1***, ***Polyporus #2*** , ***Trametes versicolor*** and ***Heterobasidium annosum***. Only the unidentified ***Basidio #1*** was a brown rotter, the remainder were white rot fungi.

Decay tests showed that ***Basidio #1*** caused the highest weight loss (22.3%) on ponderosa pine test blocks while ***Polyporus #1***, ***Polyporus #2*** and ***Trametes versicolor*** showed weight losses of 17.3%, 13.5% and 9.9%, respectively. Weight loss due to ***Heterobasidium annosum*** was only 4.9%.

Among the fungi imperfecti, ***Trichoderma***, ***Penicillium*** and ***Alternaria*** were the most frequently isolated fungi which occurred at 31.6%, 22.1% and 11.4%, respectively. These species were generally obtained from the external parts and gut of beetles. The frequency of microflora associated with male and female adults was found to be similar.

The isolated organisms were carried by beetles on or in their various body parts within depressions, sac-like structures or invaginations with setae termed as mycangia which are considered repositories of fungal spores and

mycelia. The mechanisms of transport, however, from the gut to a new host are still unclear and need further trials to determine if propagules, via this transport, can actually initiate deterioration in wood.

FUNGAL ASSOCIATES IN THEGOLDEN METALLIC BEETLE, *Buprestis aurulenta L.*

by

Carlos M. Garcia

A THESIS
submitted to
Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed September 13, 1996
Commencement June 1997

Master of Science thesis of Carlos M. Garcia presented on September 13, 1996.

APPROVED:

Signature redacted for privacy.

Major Professor, representing Forest Products

Signature redacted for privacy.

Head of Department of Forest Products

Signature redacted for privacy.

Dean of Graduate School

I understand that my thesis will become part of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Signature redacted for privacy.

Carlos M. Garcia, Author

ACKNOWLEDGMENTS

I wish to thank the following who contributed much in the completion of this project:

To Dr. Jeffrey J. Morrell, for his interest, encouragement and untiring assistance during the preparation and completion of this research project. His acceptance and approval of my application to pursue graduate studies made it possible for me to get a financial assistance under the Graduate Research Assistantship Program at the Department of Forest Products, Oregon State University (OSU).

To the Forest Products Research and Development Institute (FPRDI), Department of Science and Technology (DOST), Los Banos, Laguna, Philippines, for allowing me to pursue graduate studies abroad on official time.

To Mr. Richard L. Westcott, Entomologist, Oregon Department of Agriculture, USDA, for supplying information regarding the collection of golden metallic beetles.

To Frank Lumber Company in Mill City, OR for allowing me to collect beetles in their lumberyard in Mill City, OR.

Thanks are also due to Mr. Al Soeldner, Facility Manager, Botany and Plant Pathology Department, OSU, for his patience and assistance in the scanning microscopy of the buprestid beetle.

Assistance from Mr. Jim LaBonte, Assistant Curator of the Museum of Insects, Department of Entomology, OSU, in the determination of the sexes of the beetles, is greatly appreciated.

Identification of the microorganisms associated with the golden metallic beetle might remain unknown without the assistance of Ms. Camille Freitag. Her kindness and constructive criticism on this project are well appreciated.

To Dr. Magdalena Y. Giron, for her encouragement to continue my graduate studies at OSU. Her diligence in reviewing this manuscript is also greatly appreciated.

I also wish to thank the members of my committee for graduate studies: Drs. Darrell Ross, Tim Schowalter, Barb Gartner and Rebecca Johnson, for their comments, suggestions and recommendations in the completion of the project.

Finally, I wish to acknowledge my wife Fanny, my Princess Steph and Kuya Patrick, for their patience, love and understanding. Their moral support also made this work possible.

To all of them, this piece of work is dedicated. Thanks to everyone.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

Table **Page**

LIST OF APPENDICES

APPENDIX **Page**

LIST OF APPENDIX TABLES

APPENDIX TABLE **Page**

FUNGAL ASSOCIATES IN THE GOLDEN METALLIC BEETLE, *Buprestis aurulenta* L.

INTRODUCTION

Forest products such as logs and lumber as well as finished products from wood are subject to biodeterioration agents including insects and fungi. Losses of wood due to diseases and forest insect pests are difficult to appraise but it is estimated that 10% of the annual timber cut in the United States is used to replace wood in service (Boyce, 1961). Infestations may originate in the standing tree, but much of this damage occurs between felling and utilization. Green and seasoned lumber in the lumberyards and even the final utilized products can be attacked by a variety of insects.

Aside from being serious wood degraders, insects are efficient vectors of many important fungi. They have been reported to be involved in the transmission and development of numerous plant diseases. Vectoring provides long distance dispersal for pathogens, direct transmission to hosts, avoidance of competition with other microorganisms and sometimes creation of infection courts.

Insects are not only involved in the transmission of plant diseases but they may also co-exist with certain species of fungi wherein mutual benefits are derived from

the association. The most common example of fungal-insect interactions can be observed in the sapwood of coniferous trees. Fungi carried by bark beetles cause significant discoloration of the sapwood. These fungi do not decay the wood, but the discoloration greatly reduces the wood's market value. Blue-stain is caused by many fungi, but species of ***Ceratocystis***, ***Ophiostoma*** and related genera are the most common. The association of blue-staining fungi with Scolytidae and other bark beetles has been long known. As they emerge from infested logs, the beetles are contaminated with fungal spores both internally and externally. Conidia and ascospores of blue-staining fungi are borne in a sticky matrix in the beetle larval galleries in a way that ensures thorough contamination of the new brood of beetles. The spores of these fungi are also transported from one host to another by specialized repositories or mycangia (Batra, 1963).

One species of beetles that attack wood and cause serious damage to logs (Every and Rudinsky, 1975), and wood products (Spencer, 1930; Franz, 1936; Van Dyke, 1939) and might possibly act as vectors of some wood decaying fungi is the golden metallic beetle, ***Buprestis aurulenta*** **L.** This beetle belongs to the family Buprestidae, a group that comprises one of the largest and most destructive groups of wood attacking insects in the western United States (Every

and Rudinsky, 1975). The larvae mine in and around fire scars and mechanical injuries causing additional defects, especially in Douglas-fir and ponderosa pine.

The economic importance of golden metallic beetles in the degradation and destruction of logs and wood in service has been extensively studied. However, information as to the role of this beetle in the spread and development of wood degrading fungi is not known. Successful control strategies to eliminate or minimize insect and fungal attack on wood, with few exceptions, are dependent upon a knowledge of the organisms present. For this reason, this research project investigated the potential associations between ***B. aurulenta L.*** and wood degrading fungi as well as other microfungi.

LITERATURE REVIEW

Researchers have long known that insects are involved in the transmission and development of diseases of plants (Forbes, 1884; Waite, 1891; Stewart, 1913; Craighead, 1928). Insects studied include bees and wasps that were vectors of the bacterium causing fire blight of pears, the tarnished plant bug feeding on blighted pear twigs, and bark beetles introducing blue-stain fungi into the sapwood.

Investigators have recognized a close association between blue-staining fungi and bark beetles. These fungi do not decay the wood, but their associated discoloration greatly reduces its market value. The beetles, as they emerge from infested logs, are contaminated with fungal spores which they transport from one host to another by specialized structures in the exoskeleton of the beetles called mycangia. Given the frequent association of buprestid attack with the decayed wood, one must wonder if a similar association exists between buprestids and decay fungi as between bark beetles and blue-stain fungi.

The family Buprestidae is one of the nine families of Coleoptera that comprise the most destructive insects of forest products. The golden metallic beetle, ***Buprestis aurulenta L.***, is among the most damaging western species in this family (Every and Rudinsky, 1975). This beetle can

cause significant structural damage in log structures and utility poles.

What is the Golden Metallic Beetle?

The golden metallic beetle, ***Buprestis aurulenta L.*** is one of the most destructive species of Buprestidae (Every and Rudinsky, 1975). These beetles are active during warm sunny days and fly readily when disturbed.

The antennae have 11 segments, are short and rather slender, with fine sawlike teeth; the head is retracted into the prothorax and immaculate like the pronotum with reddish reflections. The pronotum is rigidly attached to the remainder of the body so that the wood-boring beetles, unlike the click beetles, are unable to leap into the air.

The hardened forewings (elytra) which cover the membranous hindwings are without yellow spots or markings. The protibia of male is simple. Each elytron is composed of 5-6 widely spaced costae including the short sutural costa and an apex truncate, occasionally with sutural tooth which is about 12.3-20 mm. The coxae of the hind legs are expanded into a plate that partially covers the femora and the tarsi each have five segments.

The Life Cycle

The golden metallic beetles are endopterygotes - developing from eggs to larvae, pupae and, finally to adults.

Adults lay eggs by inserting their ovipositor in cracks of fire-scorched, blazed, or injured trees (Burke, 1918) logs and windfalls (Helfer, 1941). Upon hatching, the larvae bore into wood, excavating mines which enlarges as the larvae grow in size. The larvae first bore in the phloem region of the trunk and branches, then penetrates and mines the wood extensively. The larvae leave oval galleries tightly packed with frass. The mines are oval in cross section and about 9 mm in diameter. Larval tunnels can extend from 0.9 to 4.5 m in length.

The full grown larvae measure about 37 mm long. Larval growth continues until fall, when activity ceases with the advent of cold weather. Larval development requires from 1 to 4 years (Linsley, 1943; Chamberlain, 1947).

The larvae enter into a pupal stage which occurs near the surface of the wood and molt into adult beetles. Metamorphosis occurs in late summer (Burke, 1918) or fall (Helfer, 1941) and the adult overwinters in the pupal cell to emerge during the following spring (Linsley, 1943).

The iridescent, golden- or blue-green colored adult which measures 14-21 mm in length (Garnett, 1918), excavates its way out of the wood, leaving a small oval exit hole (Chamberlain, 1949) which is often the first evidence of an infestation. Adults of both sexes require a period of feeding on foliage for maturation of reproductive organs. However, females feed more heavily than males with the most active females being the heaviest feeders and the most active males being the lightest feeders (Smith, 1962). Adult males have been reported to have a longer life span than females averaging 120 days and 109 days, respectively.

There are reports of larval development extending for prolonged periods in some wood products (Smith, 1962). Adults have been reported to emerge from varnished handrails installed nine years earlier (Chamberlin, 1924); from a 26-year-old house in Oakland, California (Huguenin, 1915); from various structures after 14 to 20 years in British Columbia (Spencer, 1930) and from a fixture in an Oakland, California building after 30 years (Van Dyke, 1939). Franz (1936) reported that ***B. aurulenta L.*** had emerged from a 13-year-old North American wood dresser in Frankfurt, Germany. The Victoria Laboratory project file includes an annotated list of 120 cases of property infestation by ***B. aurulenta L.*** in British Columbia (Smith, 1962).

Host Range

Buprestis aurulenta L. occurs throughout the range of its primary host, Douglas-fir, ***Pseudotsuga menziesii*** (Burke, 1918). This beetle also attacks ***Thuja plicata***, ***Abies grandis***, ***Pinus ponderosa***, ***P. jeffreyi***, ***P. lambertiana***, ***P. contorta*** , ***P. flexilis***, and ***Picea sp.*** (Garnett, 1918).

Distribution

The golden metallic beetle is widely distributed in the Pacific Coast and Rocky Mountain states as well as parts of Canada. It is abundant in British Columbia, Washington, Oregon, and California, and less common in Montana, Idaho, Wyoming, Colorado, New Mexico, Utah, Arizona and Nevada (Helfer, 1941; Chamberlin, 1949).

The adults may appear in March (Chamberlin, 1924) but more commonly in April. They are active until September with peak emergence between May and June (Burke, 1918). From April through the summer, beetles can be collected from the foliage of young pines (Helfer, 1941).

The Wood-Inhabiting Fungi

Fungi, like insects, are among the major groups of biotic agents that deteriorate wood between felling and

final utilization. Wood degrading fungi include molds, stain and decay fungi that affect wood in storage or *in situ*.

Molds are fungi that grow extensively on extremely wet sapwood surfaces, utilizing available simple carbohydrates. The discoloration results from the masses of pigmented hyphae on the wood surface. Staining fungi cause abnormal discoloration of wood and primarily colonize parenchymatous tissues in sapwood.

Decay fungi utilize the structural components of the wood causing significant reductions in wood strength properties. Brown rot fungi metabolize the carbohydrates, leaving a partially degraded lignin (Hartig, 1947). White rotters degrade both carbohydrates and lignin, while soft rot fungi attack carbohydrates in the secondary cell wall layers forming cavities or eroding the secondary cell wall.

Beetle-Fungus Interactions

The range of possible interactions between insects and fungi have been well documented. These relationships may include mycophagy, mutualism, mycopathogens of insects or vectoring of fungal diseases (Wilding et al., 1989).

Mycophagy is common in the Coleoptera and refers to the dependence of the insect on fungal mycelia, fruiting

structures or spores for some or all of its nutrition. Swift and Boddy (1984) reported that fungal hyphae provide a richer source of protein than dead wood. This protein is essential for beetle development.

In mutualism, each of the partners gains by the association. For example, the ambrosia fungus depends on the ambrosia beetles for dispersal while the ambrosia beetle larvae depend on the growth of ambrosia fungus for their nutrition (Wilding et al., 1989).

Mycopathogen-insect interactions involve the invasion or colonization by fungal spores or mycelia resulting in the mortality of the insect. This is best demonstrated by the many entomogenous fungi that are used as biological control agents of insect pests. For example, ***Beauveria bassiana***, an entomopathogenic hyphomycete, decreased total food consumption of the fourth-instar Colorado potato beetles, ***Leptinotarsa decelineata*** (Say) by 76% (Fargues et al., 1994). Likewise, introduction of this pathogen into the soil resulted in increased mortality of the adults, retarded emergence in the spring, and reduced viability of Colorado beetle populations in later generations (Bajan et al., 1977).

Insects As Vectors of Fungal Diseases

Insects are efficient transmitters of plant diseases and their significance as disease carriers has long been recognized (Waite, 1891). Insects are successful disease carriers because they provide long distance transport, direct transmission to hosts, avoidance of competition with other microorganisms, and creation of infection courts (Purcell, 1982a). However, other factors such as coincidence of insect-pathogen life cycles, survival of spores during transport and amount of inoculum transported can markedly influence disease transmission (Webber and Gibbs, 1989). Although insects are important in the spread of only a few fungal pathogens, very few viable spores are needed to initiate infection (Ingold, 1978).

Bark beetles are well established "transmitters" of pathogens (Webber and Gibbs, 1989). For instance, the Dutch elm disease caused by ***Ophiostoma ulmi*** is vectored by the bark beetles ***Scolytus multistriatus*** and ***Hylurgopinus rufipes*** (**S. *kirchie*** and ***S. laevis*)** (Lekander et al., 1977). Oak wilt caused by ***Ceratocystis fagacearum*** is transmitted by nitidulid beetles (Gibbs and French, 1980) and the black stain root disease of Douglas-fir caused by ***Leptographium wageneri*** var. ***pseudotsugae*** (Cobb, 1988; Webber and Gibbs, 1989) is vectored by ***Pissodes fasciatus***, ***Steremnius***

carinatus, and one scolytid, ***Hylastes nigrinus*** (Witcosky et al., 1986; Goheen and Hansen, 1993).

Fungi Associated with Ambrosia Beetles and Other Beetle Species

Members of the genus ***Ceratocystis*** are often mutualistically associated with bark beetles (Baker, 1963; Batra, 1967; Francke-Grosmann, 1967; Kok, 1979; Norris, 1979; Whitney, 1982). ***Ceratocystis minor*** (Hedgecock) Hunt is a pathogenic blue-stain fungus frequently associated with several species of bark beetles, including the southern pine beetle (Craighead, 1928; Nelson and Beal, 1929; Rumbold, 1931). The earliest suggestion of an association of insects with blue-stain fungi was reported by von Schrenk (1903). Tree death caused by blue-stain fungi vectored by bark beetles has also been reported (Nelson and Beal, 1929).

A variety of Ascomycetes and yeasts, especially ***Penicillium*** and stain fungi, ***Ophiostoma***, have been isolated from ambrosia beetles, bark beetles and wood borers. These insects were collected from experimental boles as was ***Heterobasidium annosum*** (Schowalter et al., 1992).

The primary benefit of an insect association to the fungus is reliable spore dispersal and inoculation into new

habitats suitable for colonization. On the other hand, the fungi form the only real source of food for ambrosia beetle adults and larvae (Norris and Baker, 1967). Fungal hyphae provide a richer source of protein than dead wood and fungi are able to concentrate nitrogen from substrates where this element occurs at very low levels (Swift and Boddy, 1984). Some fungi also help to reduce host tree defenses and improve the chances of beetle colonization (Berryman, 1972, 1989). Aside from nutritional benefits (Norris and Baker, 1967; Kok et al., 1970), fungi may also stimulate beetle aggregation (Brand et al., 1976; Brand and Barras, 1977). Some ***Dendroctonus*** and ***Scolytus*** species which attack living conifers are often found in close association with specific fungi that may assist in aggregation (Crowson, 1984).

Many species of bark beetles succeed in their host colonization by introducing fungal hyphal masses (Graham, 1967; Reid et al., 1967; Barras and Perry 1971, 1972; Berryman, 1972; Wood, 1982; Bridges and Master, 1983). These beetles have special spore repositories and may produce special secretions to promote spore adhesion and viability until the propagules can be inoculated in a new host tree (Happ et al., 1971).

Other fungi and bacteria are also associated with the beetles and their galleries (Whitney, 1982). Many species, e.g., ***Penicillium spp.*** and ***Trichoderma spp***, seem to be

"weed" fungi with no more than a commensal relationship with the beetles. They may sometimes be used as a supplementary food source (Baker, 1963), but tend to occur in old or abandoned galleries.

Neger (1911) considered transmission of ambrosia fungi to the new host in the gut of the beetle. A shell of dried spores and mucilage protects the spores against desiccation and UV exposure (Dowding, 1969), and from digestion in the gut of the beetle (Francke-Grosmann, 1963). However, Done and Gilliland (1929) suggested transmission by adhesion of fungal spores to the exoskeleton of the beetle. Barras and Perry (1972) also suggested that the spores were carried between trees on the external body surface of the beetle.

Nunberg (1951) suggested that tubelike glands located in the pronotum of female ***Trypodendron spp.*** play a role in fungal transmission. This finding was confirmed by Francke-Grosmann (1956) in the same species and various other Scolytidae and Platypodidae. The term mycangia was suggested to describe such organs (Batra, 1963).

The methods of transmission and the locations of the specialized structures used to transport spores from one host to another vary among bark beetle species. Some Scolytids (***Pityoborus comatus***) and Platypodids (***Platypus wilsoni***) carry mutualistic fungi in repositories in their

integument (Francke-Grosmann, 1963; Furniss et al., 1987). These repositories may occur on the thorax, head and mouthparts, or elytra. Fungal spores were abundant in the female-specific pubescent pronotal depressions on four ***Pityoborus spp.*** (Furniss et al., 1987).

The southern pine beetle, ***Dendroctonus frontalis*** Zimmerman, lives in association with two symbiotic fungi that are propagated and transported in the adult female's prothoracic mycangium (Barras and Perry, 1972). These fungi are obligate symbionts, and their absence results in poor development of beetle larvae. Spores of the blue-stain fungus are not carried in the mycangium but are carried on the beetle's exoskeleton or by phoretic mites. Either a single species occupies the whole mycangium, or the two species occur on opposite sides of the mycangium (Barras and Taylor, 1973) and are seldom intermixed (Barras and Perry, 1972).

Levieux et al. (1991) reported that the European bark beetle, ***Ips sexdentatus*** Boerner, carried fungi in puncture pits located on the proximal part of each mandible, the sides of the pronotum, and the elytra. Thus, it appears that this beetle has evolved a mechanism for the protection and dissemination of yeast and fungi such as ***Ceratocystis spp.***

Research on Buprestid-Fungal Association

There are numerous reports of insect-fungi associations. Relatively little is known about the relationships between buprestids and decay fungi, probably because of the beetle's long life cycle. It is surprising, however, that there is only limited information about the economic importance of golden buprestid beetle since this species is reported to be among the most destructive species of the Buprestidae. Stephen and co-workers (1993) reported that the genus ***Buprestis*** was associated with ***Dendroctonus***, ***Scolytus*** and ***Ips*** bark beetles on conifers in North America. Members of the Buprestidae are among the phloem-inhabiting species that rapidly locate and colonize the nutrient rich tissue of freshly killed conifers .

Buprestidae are mainly wood decomposers. However, Edmonds and Eglitis (1989) reported them as vectors of wood-decomposing fungi and showed that their exclusion from logs slows decomposition significantly. Their research however, did not provide any information to assess the probable role of buprestids in fungal dissemination. These beetles are abundant in log decks at many Pacific Northwest lumber mills and this prevalence raised questions concerning the possible role of this beetle as a vector of decay fungi. The determination of the identities of the

associated microorganisms is a critical step in the control of most disease and biodeterioration problems (Zabel and Morrell, 1992).

OBJECTIVE

The objective of this research was to determine the role of ***Buprestis aurulenta L.*** as a potential vector for basidiomycetes and other microfungi in freshly fallen timber. This overall objective was addressed by collecting beetles from different geographic locations in western Oregon and isolating associated fungi from various body parts. Fungi isolated in this manner were assessed for their ability to cause deterioration of wood.

MATERIALS AND METHODS

Collection of Golden Metallic Beetles

Adult beetles were collected at four sawmill and lumberyards located in western Oregon. Collections were attempted at the Georgia Pacific Company (Philomath, OR); Green Veneer Inc. (Idanha, OR); Young and Morgan Company (Mill City, OR); and Frank Lumber Company (Mill City, OR). No beetles were found on the log decks of the first three companies.

Collection of the beetles in the four lumberyards was performed in July and August of 1996. Sterile screw capped vials (70 mm x 20 mm in diameter) were used to trap the adult beetles on the cut-ends surface of the logs. Each vial containing an adult beetle was loosely capped after the insect was trapped. The vials were brought to the laboratory for subsequent isolation of microorganisms associated with the beetle. Collections were generally performed between mid-morning and afternoon, reflecting the beetle's habit of migrating more frequently during warm, sunny days.

Isolation of Fungal Associates From the Golden Metallic Beetle

Each beetle was surface-sterilized by passing briefly over a flame. Only live, active beetles were used. Each beetle was sexed prior to the isolation process. The sex was determined by examining the posterior abdominal portion and configuration of the last abdominal sternum of the beetle. The female has an ovipositor and the last abdominal sternum is emarginate and slightly rounded. In contrast, the male has an aedaegus and the last abdominal sternum is straighter and not rounded (Hatch, 1971). A total of 80 golden metallic beetles were used in the isolation studies. Twenty insects were randomly allocated for each of the following isolation methods:

Isolation from the External Surfaces of the Beetle

The legs, head, thorax and elytra were cut from 10 live beetles using a sterile razor and scissors. Each insect part was planted separately on 1% malt extract agar (MEA) in plastic petri dishes. An additional 10 beetles were prepared in the same manner but the parts were planted on MEA amended with 10 ppm benomyl to retard the growth of non-basidiomycetes.

Isolation from the Internal Part of the Beetle

Beetles were mounted on paraffin surfaces and dissected with sterile scissors and disposable razor blades. The abdominal integuments were cut from the foregut to the aedaegus for the male or including the ovipositor for the female. The whole gut was directly planted on plated MEA or MEA plus benomyl. Ten beetle guts were sampled per culture medium.

Crawling of Adult Beetles on Culture Media

Adult beetles were allowed to crawl on the surface of plated malt agar media with or without benomyl for 30 minutes. Ten beetles were also evaluated on each culture medium.

Isolation from Washings of Adult Beetles

Ten ml of sterile distilled water was poured in a vial containing a live adult beetle and allowed to stand for 10 seconds. The liquid was then shaken prior to removal of the insect from the vial. A sterile wire loop was dipped in the water and streaked on the surface of MEA or MEA plus benomyl media. The test was replicated using 10 beetles per media.

All of the inoculated plates were incubated at 28 °C and observed daily for evidence of microbial growth. Any mycelium growing from the body parts or streaked water on the surface of the plated media were subcultured onto fresh media for subsequent characterization and identification purposes.

The frequency of occurrence of the various isolates was computed as follows:

$$\% \text{ Frequency of Isolates} = \frac{\text{No. of Isolates per Genus}}{\text{Total Number of Isolates}} \times 100$$

Distribution of Basidiomycetes, Fungi Imperfecti and Ascomycetes on Female and Male Adult Golden Metallic Beetles

The differences in the number of fungi isolated from female and male golden metallic beetles were determined by Wilcoxon Rank Sum Test (Ramsey and Schafer, 1994) using the SAS software (Cody and Smith, 1991).

Characterization and Identification of Fungi Associated with Golden Metallic Beetle

All fungal isolates were characterized based on their color and nature of growth in the culture media. Fungi were observed microscopically to determined the morphological features, i.e., shape and size of spores,

hyphae and other pertinent structures. Sections were stained with lactophenol in cotton blue or with phloxin red dye. Diagrams were drawn for each isolate and were compared with previously identified or known cultures (Nobles, 1940; Barron, 1962; Ainsworth, 1966; Ellis, 1971; 1976; Ramirez, 1982).

For cultures that were suspected to be wood decayers, the isolates were examined for the presence of clamp connections. The ability of the isolate to cause decay in wood was also determined and the isolates were grown in gallic or tannic acid media to determine whether they were brown or white rot fungi (Davidson et al., 1938; Nobles, 1948).

Microscopic Examination of Decay Fungi

Five- to 7-day-old cultures of decay fungi were examined under the microscope. Characteristic features of the hyphae, spores or special structures such as cystidia and setae and formation of fruiting bodies were observed (Nobles, 1948).

Ability of Fungi to Cause Decay

The ability of the isolates to cause wood degradation was assessed using a modified soil block test (ASTM D 2017-

81, 1986; Scheffer et al., 1987). Fifty-six ml glass bottles were half-filled with forest loam soil. A moistened 10 mm x 10 mm x 3 mm thick ponderosa pine (***Pinus ponderosa***) feeder strip was placed on the soil surface. The soil was moistened to approximately 60% of field capacity, then the jars were capped loosely and autoclaved for 45 minutes at 121 °C and 15 psi. The jars with feeder strips were allowed to cool off for 24 hours before heating for another 15 minutes at 121 °C to assure complete sterilization. After cooling, a 3 mm diameter agar plug cut from the actively growing edge of the test fungus was placed on the edge of the feeder strip and the jars were incubated at 28 °C until the feeder strip was thoroughly colonized by the fungus. Jars inoculated with standard test fungus, ***Postia placenta***, and uninoculated jars with test wood blocks were included for comparison. An irradiated (2.5 mrad) ponderosa pine sapwood block wafer (1.0 cm^3) which was previously oven-dried (54 °C) and weighed (nearest 0.001 g) was individually introduced per decay chamber. The wafers were incubated for 12 weeks at 28 °C. The wafers were removed, cleaned of any adhering mycelia on the block surface, oven-dried to constant weight, and reweighed to assess wood weight loss due to fungal exposure. Differences between initial and final

wood weight were used to calculate percentage wood weight loss.

Growth on Gallic or Tannic Acid Culture Media

Cultures of potential wood decaying fungi were subjected to a reaction test on culture media containing tannic or gallic acid. The intent of this test was to describe the characters of the cultures when grown on malt agar and malt agar containing gallic or tannic acids (Davidson et al., 1938).

Malt agar culture medium was prepared as follows: Five g of agar and 7.5 g of malt extract were dissolved in 500 ml of distilled water and sterilized for 20 minutes at 121 °C and 15 psi.

Gallic or tannic acid agar medium was prepared as follows: Twenty g of agar and 15 g of malt extract were dissolved in 850 ml of water. Another 150 ml of water was placed in a separate flask. Both flasks were sterilized for 20 minutes at 121 °C and 15 psi. While the sterilized water was still hot, 5 g of gallic or tannic acid were dissolved in it. This solution was added to the slightly cooled malt agar and thoroughly mixed before being poured directly into sterile disposable petri dishes.

The potential wood decaying fungi were grown individually in petri dishes for 7 days. From this actively growing culture, a plug of 3 mm diameter was taken and inoculated aseptically on the center of gallic, tannic or plain malt agar culture medium. The petri dishes were incubated at 30 °C and were examined after 1 and 2 weeks. The rate of growth, form and character of the advancing zone, color and reaction on media were recorded. The growth and response of the microorganisms to the culture media were assessed using the criteria described in Appendices 1 and 2.

Examination of Morphological Features on the External Parts of Adults of Golden Metallic Beetle

The presence of specialized structures on or in the exoskeletons of buprestid beetle which could possibly transport propagules were examined. A pair of adult male and female beetles were studied using a scanning electron microscope.

The test insect was mounted on an insect pin and air-dried. The pinned specimen was fastened to an aluminum SEM mount using copper-backed adhesive tape. The specimen was coated with 200 NM of gold in an Edwards S150B sputter coater at 1 x 10^{-2} Torr. The body and appendages of the specimen were examined using an AmRAY 1000A Scanning

Electron Microscope operated at an accelerating voltage of 7 kV, in the Electron Microscope Facility, Department of Botany and Plant Pathology, OSU. Images were recorded on Polaroid Type 55 P/N 4 x 5" format film.

RESULTS AND DISCUSSION

Collection of Golden Metallic Beetles

A total of 80 adult golden metallic beetles were collected from the Frank Lumber Co. in Mill City, Oregon. The log stock was 90% Douglas-fir, the primary host of ***B. aurulenta L.*** (Burke, 1918) and 10% other coniferous species, which are likewise attacked by this beetle (Garne, 1918). The presence of a large volume of logs in the lumberyard and the year round availability of the primary host, made this site ideal for the breeding and collection of ***B. aurulenta L.***

Among the golden metallic beetles collected, 21 were males and 59 were females. Male beetles were observed courting the females on the logs. The high frequency of the female population was probably due to the fact that collection coincided with the egg-laying season of the beetles. The females were seen on the cut ends of the logs with their ovipositor out swabbing left and right, looking for oviposition sites. In some instances, the females stood still on the cut ends of the logs with their ovipositor inserted inside the crevices.

The adults were observed emerging from between the bark and the sapwood of logs in the old piles between 10

o'clock in the morning and 4 o'clock in the afternoon during warm, sunny days. The insects were absent during cloudy and windy days. The beetles would crawl or momentarily stay on the surface of the old stock log before migrating to the new piles in the log deck. Swarming of the beetles was also observed during the debarking of the logs in the lumberyard. Garnett (1918) also reported that adult beetles were attracted to freshly sawn wood for several weeks in July. The pitchy wood of Douglas-fir appeared to attract males and females, for they were frequently seen in newly piled logs. The same was observed by Smith (1962) who found larvae to be associated with pitchy wood.

On several occasions, mating adults were noted on the cut ends of the logs in the new pile. Likewise, females were observed laying eggs by inserting their ovipositors in the cracks or crevices of the cut ends. Adult female beetles which were allowed to oviposit between a glass slide and Douglas-fir wood block in the laboratory were observed to lay an average of 20 eggs (Fig. 1). The average incubation period of the eggs was around 15 days.

Older logs where emerging beetles were observed showed some discoloration patterns on the cut ends and sapwood suggesting that the wood had remained in the deck for some time.

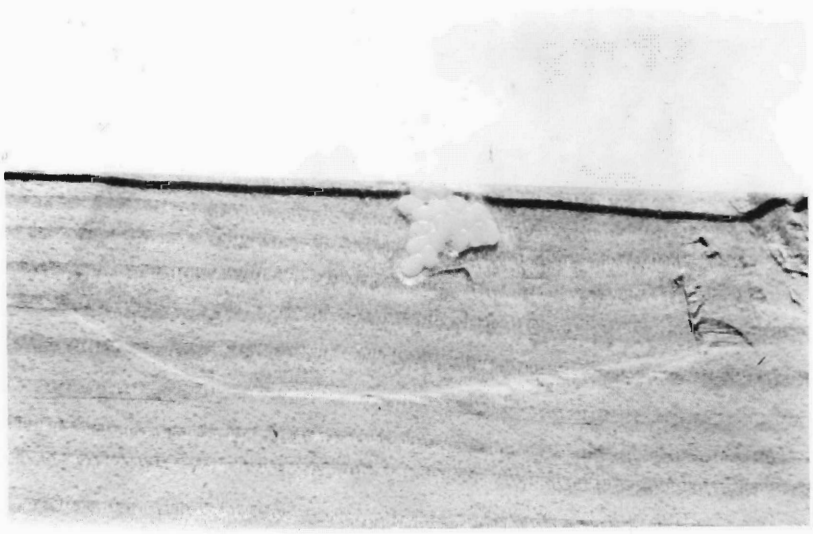

Fig. 1. Two-Day-Old Eggs of a Golden Metallic Beetle Laid Between a Douglas-fir Wood Block and a Glass Slide.

No beetles were collected from the lumberyards of Georgia Pacific (Philomath, OR) and Green Veneer Inc. (Idanha, OR) after 3 days of collection. This probably reflects a limited stock of logs and immediate processing of logs within a week after delivery in both mills. Although the lumberyard area and the volume of stock piles at Young and Morgan Company were almost the same as the Frank Lumber Co., no beetles were also found after 6 collection trips in the area. There was a continues application of water on the log decks at this mill and this moisture probably limited the access of ***B. aurulenta L.***

Frequency of Distribution of Fungal Isolates of Adults of Golden Metallic Beetle

A total of 863 fungal isolates were obtained from the 80 golden metallic beetles examined using the four methods of isolation (Table 1). These isolates comprised four genera of Basidiomycetes, 24 genera of Fungi Imperfecti and one genus of Ascomycetes.

These fungi were frequently isolated from the external parts which included the legs, head, thorax, elytra, and also from the internal gut of the beetle. Regardless of the genus, most of the fungi were isolated from the external parts (54.8%), while 19.6% came from the gut, 13.6% by crawling and 12.1% were obtained by the streaking

Table 1. Fungi Isolated from Various Body Parts of Adults of Golden Metallic Beetles.

Fungi	Number of Isolations								
	Crawling	Streaking	Gut	External				Total	Grand
				Legs	Head	Thorax	Elytra	*External*	Total
Basidiomycetes									
Polyporus #1	3	0	0	1	0	1	4	6	9
Polyporus #2	1	1	0	0	2	1	0	3	5
Heterobasidium	0	1	3	1	0	0	0	1	5
Basidio #1	0	2	0	1	0	0	0	1	3
Trametes	1	0	0	1	0	0	0	1	2
Fungi Imperfecti									
Trichoderma	50	17	35	66	29	30	46	171	273
Penicillium	24	35	64	24	13	15	16	68	191
Alternaria	5	0	20	18	10	18	27	73	98
Cladosporium	3	11	6	13	4	2	6	25	45
Aspergillus	4	1	5	18	2	4	2	26	36
Neurospora	8	0	0	13	3	0	1	17	25
Scytalidium	3	7	4	2	1	0	6	9	23
Dreschlera	2	0	2	2	4	4	4	14	18
Rhizopus	1	1	3	4	0	2	0	6	11
Curvularia	0	0	0	5	1	1	3	10	10
Aureobasidium	1	4	1	1	0	0	1	2	8
Torula	1	1	0	0	0	3	2	5	7
Nigrospora	0	0	1	0	2	2	2	6	7
Gliomastix	1	2	0	1	1	1	0	3	6
Rhinocladiella	0	0	3	1	1	0	1	3	6
Ulocladium	0	0	0	1	0	2	2	5	5
Torulomyces	0	1	1	0	1	0	1	2	4
Epicoccum	0	0	0	0	1	2	0	3	3
Graphium	0	1	0	0	1	0	0	1	2
Botrytis	1	0	0	0	0	0	0	0	1
Stachybotrys	1	0	0	0	0	0	0	0	1
Unidentified Imperf. 1	0	0	1	0	1	0	0	1	2
Unidentified Imperf. 2	0	1	0	2	0	3	1	6	7
Unidentified Imperf. 3	1	4	3	0	0	2	1	3	11
Ascomycetes									
Yeasts	6	14	17	0	0	1	1	2	39
Sub-total	117	104	169	175	77	94	127	473	863
% Freq. Based on Methods	13.6	12.1	19.6	20.3	8.9	10.9	14.7	54.8	100.0

method. The fungal isolates from the external body parts occurred most frequently from the 3 pairs of legs (20.1%), followed by the elytra, the thorax and the head.

Basidiomycetes were observed at low levels, representing 0.2 to 1.0% of the total isolated fungi (Table 2). ***Polyporus #1*** was the most frequently isolated basidiomycete occurring in 1.0% of the isolations, followed by ***Polyporus #2*** and ***Heterobasidium annosum*** with a frequency of 0.6%. An unidentified basidiomycete coded as ***Basidio #1*** and ***Trametes versicolor*** were observed at 0.4% and 0.2%, respectively, of isolations from the legs of the insect, streaking of washings, or crawling of the beetle on the agar surface.

The occurrence of basidiomycetous fungi at a low frequency has been similarly obtained from experimental conifer bolts in a study conducted by Schowalter et al. (1992). ***Heterobasidium annosum*** was present in less than 1.0% of isolations from bark, ambrosia and wood borer beetles.

Among the Fungi Imperfecti, ***Trichoderma*** were the most frequently isolated fungus, comprising 31.6% of the total isolates (Table 2). This genus was followed by ***Penicillium*** and ***Alternaria*** representing 22.1% and 11.4%, of the isolates, respectively.

Table 2. Frequency of Distribution of Fungal Isolates from Various Body Parts of Adults of Golden Metallic Beetles.

Fungi	Frequency of Isolation								
	Crawling	Streaking	Gut	External				Total External	Total Freq.
				Legs	Head	Thorax	Elytra		
Basidiomycetes									
Polyporus #1	33.3	0.0	0.0	11.1	0.0	11.1	44.4	66.7	1.0
Polyporus #2	20.0	20.0	0.0	0.0	40.0	20.0	0.0	60.0	0.6
Heterobasidium	0.0	20.0	60.0	20.0	0.0	0.0	0.0	20.0	0.6
Basidio #1	0.0	66.7	0.0	33.3	0.0	0.0	0.0	33.3	0.3
Trametes	50.0	0.0	0.0	50.0	0.0	0.0	0.0	50.0	0.2
Fungi Imperfecti									
Trichoderma	18.3	6.2	12.8	24.2	10.6	11.0	16.8	62.6	31.6
Penicillum	12.6	18.3	33.5	12.6	6.8	7.9	8.4	35.6	22.1
Alternaria	5.1	0.0	20.4	18.4	10.2	18.4	27.6	74.5	11.4
Cladosporium	6.7	24.4	13.3	28.9	8.9	4.4	13.3	55.6	5.2
Aspergillus	11.1	2.8	13.9	50.0	5.6	11.1	5.6	72.2	4.2
Neurospora	32.0	0.0	0.0	52.0	12.0	0.0	4.0	68.0	2.9
Scytalidium	13.0	30.4	17.4	8.7	4.3	0.0	26.1	39.1	2.7
Dreschlera	11.1	0.0	11.1	11.1	22.2	22.2	22.2	77.8	2.1
Rhizopus	9.1	9.1	27.3	36.4	0.0	18.2	0.0	54.5	1.3
Curvularia	0.0	0.0	0.0	50.0	10.0	10.0	30.0	100.0	1.2
Aureobasidium	12.5	50.0	12.5	12.5	0.0	0.0	12.5	25.0	0.9
Torula	14.3	14.3	0.0	0.0	0.0	42.9	28.6	71.4	0.8
Nigrospora	0.0	0.0	14.3	0.0	28.6	28.6	28.6	85.7	0.8
Gliomastix	16.7	33.3	0.0	16.7	16.7	16.7	0.0	50.0	0.7
Rhinocladiella	0.0	0.0	50.0	16.7	16.7	0.0	16.7	50.0	0.7
Ulocladium	0.0	0.0	0.0	20.0	0.0	40.0	40.0	100.0	0.6
Torulomyces	0.0	25.0	25.0	0.0	25.0	0.0	25.0	50.0	0.5
Epicoccum	0.0	0.0	0.0	0.0	33.3	66.7	0.0	100.0	0.3
Graphium	0.0	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.2
Botrytis	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Stachybotrys	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Unidentified Imperfect 1	0.0	0.0	50.0	0.0	50.0	0.0	0.0	50.0	0.2
Unidentified Imperfect 2	0.0	14.3	0.0	28.6	0.0	42.9	14.3	85.7	0.8
Unidentified Imperfect 3	9.1	36.4	27.3	0.0	0.0	18.2	9.1	27.3	1.3
Ascomycetes									
Yeasts	15.4	35.9	43.6	0.0	0.0	2.6	2.6	5.1	4.5
									100.0

Although ***Trichoderma***, ***Penicillium*** and ***Alternaria*** were frequently isolated from the external parts, these fungi were also obtained from the gut of the beetle. ***Penicillium*** isolates represented 33.5% of all isolation from the gut, while ***Alternaria*** and ***Trichoderma*** represented 20.4% and 12.8%, respectively, of these isolations.

Cladosporium, ***Aspergillus***, ***Neurospora***, ***Scytalidium***, ***Dreschlera*** and ***Rhizopus*** were considerably less frequent, occurring at 1.3% to 5.2%. Except for ***Neurospora***, these fungi can cause blue to black discoloration of wood (Wang and Zabel, 1990; Zabel and Morrell, 1992). These fungi have been reported to be commonly associated with other beetles (Baker, 1963; Whitney, 1982; Carpenter et al., 1988; Schowalter et al., 1992) causing staining or discoloration of sapwood in coniferous trees. Other fungi including ***Curvularia***, ***Aureobasidium***, ***Torula***, ***Nigrospora***, ***Gliomastix***, ***Rhinocladiella***, ***Ulocladium***, ***Torulomyces***, ***Epicoccum***, ***Graphium***, ***Stachybotrys***, and ***Botrytis*** were likewise obtained at extremely low levels ranging from 0.1% to 1.2%. Three unidentified isolates occurred at 0.2%, 0.8% and 1.3% frequency. Yeasts were frequently isolated from the gut of the beetle and represented 4.5% of the fungal population obtained.

Only 2.8% of the total isolates obtained from the beetles were Basidiomycetes, 92.7% were Fungi Imperfecti and 4.5% were Ascomycetes (Table 3). Basidiomycetes and Fungi Imperfecti were mostly isolated from the external parts. For Basidiomycetes, the number of isolates obtained from the external parts were similar to the total obtained from crawling, streaking and the gut portion of the beetle.

The presence of fungi in the gut of the beetle raises some interesting questions. It is unknown whether these isolations represent inadvertent uptake of spores or hyphal fragments during normal feeding or a more defined association. The transmission of the fungi via the gut would require a study to assess uptake, survival and the ability of the propagules to initiate colonies in other wood samples.

Likewise, Fungi Imperfecti were generally isolated from external parts with 53.2% coming from the legs, head, thorax and elytra. Yeasts were isolated at a higher rate from the gut (2.0%) followed by streaking of washings from the beetle's body (1.6%). A small proportion of the isolates was obtained from the beetle crawling on the surface of the culture media.

Table 3. Frequency of Basidiomycetes, Fungi Imperfecti and Ascomycetes Isolated from Adults of Golden Metallic Beetles by Various Methods.

Method	Basidiomycetes		Imperfect Fungi		Ascomycetes	
	No.	%	No.	%	No.	%
Crawling	5	0.6	106	12.3	6	0.7
Streaking	4	0.5	86	10.0	14	1.6
Gut	3	0.3	149	17.3	17	2.0
External	12	1.4	459	53.2	2	0.2
Legs	4	0.5	171	19.8	0	0.0
Head	2	0.2	75	8.7	0	0.0
Thorax	2	0.2	91	10.5	1	0.1
Elytra	4	0.5	122	14.1	1	0.1
	24	2.8	800	92.7	39	4.5

Distribution of Basidiomycetes, Fungi Imperfecti and Ascomycetes on Female and Male Golden Metallic Beetles

The total number of fungi isolated from the 59 female beetles examined was 622 while 241 isolates were obtained from 21 male beetles (Table 4). Analysis of the difference in the frequency distribution of each class of fungi using the Wilcoxon Rank Sum Test indicated that there was no significant difference in the average number of Basidiomycete isolates per beetle between the sexes (Appendix 3). The probability that the Basidiomycetes isolated between the female and male beetle differed by chance alone was 0.3658, which is greater than the p-value of 0.05 suggesting that there was no significant difference in the number of isolates that could be obtained from female and male beetles. Likewise, no significant differences were noted between the associations of Fungi Imperfecti or Ascomycetes on male and female buprestids (Appendices 4 and 5).

A variety of species were present on and in the beetles, but the mechanism of transport of these propagules and the potential roles of these fungi in colonization or utilization of the wood are unclear.

Table 4. Distribution of Basidiomycetes, Fungi Imperfecti and Ascomycetes on Female and Male Adult Golden Metallic Beetles.

Fungal Group	Number of Isolates	
	Females	Males
Basidiomycetes	17	7
Fungi Imperfecti	580	220
Ascomycetes	25	14
Total No. of Isolates	622	241

Growth and Reactions of Isolated Basidiomycetes on Gallic or Tannic Acid Culture Media

Of the five basidiomycete isolates that were tested for growth and reactions to gallic and tannic acid media, four were identified as white rot fungi. These were ***Polyporus #1***, ***Polyporus #2***, ***Trametes versicolor*** and ***Heterobasidium annosum***. ***Basidio #1*** decay fungus was determined to be a brown rotter (Table 5).

The average growth of the ***Basidio #1*** was 85 mm on malt agar and 44 mm on gallic acid. The fungus did not grow on tannic acid media and showed a negative reaction to both culture media. Mycelia of the fungus was thin, radiating from the center towards the margin of the colony, becoming cobwebby and tending to form small, scattered mycelial clumps at maturity. Hyphae were simple with branched staghorn aerial hyphae. Fiber cells were also observed on mature cultures.

Growth of ***Polyporus #1*** on malt agar was 85 mm on malt agar; 75 mm on gallic acid and none on tannic acid media. It showed a positive reaction to both media. Mycelium was thin, white, almost transparent, appressed and radiating from the center towards the margin of the fungal mat. At maturity, small nodes or clumps measuring about 2 mm were observed to be generally submerged in the substrate.

Table 5. Growth and Reactions of Selected Basidiomycetes on Gallic and Tannic Acid Culture Media.

Fungi	Growth (mm) 1/			: Reaction 2/		
	Malt Agar	Gallic	Tannic	: Gallic	Tannic	Decay Type
Basidio #1	85	44	0	: -	-	brown rot
Polyporus #1	85	75	0	: +	+	white rot
Polyporus #2	85	0	25	: +	+	white rot
Trametes versicolor	85	0	32.5	: +	+	white rot
Heterobasidium annosum	15.5	0	0	: +	+	white rot

1/ Average of 2 replicates of growth in mm of basidiomycetes in culture media after 2 weeks of incubation.

2/ - Negative reaction, no oxidation or brown discoloration of the culture media.

+ Positive reaction, present of oxidation or brown discoloration of the culture media.

Hyphae were simple, branched but some were with thick-walled cells.

***Polyporus* #2** grew rapidly (85 mm) on malt agar but slowly (0 and 25 mm) on gallic and tannic acid media after 2 weeks, respectively. Growth showed a positive reaction to both media. The mycelium was thin and white and the fungal mat had fine radiating lines from the center towards the margin. It formed bunches of white tuft mycelia at maturity at the center and edge of the plate. Hyphae were simple and simple setae were observed.

Trametes versicolor showed a moderately strong positive reaction to both gallic and tannic acid media exhibiting dark brown diffusion zones. The fungus did not grow on the former, but was 32.5 mm in diameter on the latter culture media. However, the growth was rapid on regular malt agar attaining 85 mm diameter in 2 weeks. Aerial mycelia were present which turned felt-like with exudate at maturity. It had a characteristic odor and the reverse side of the plate was bleached.

Heterobasidium annosum showed strong positive reaction to gallic and tannic acid media without the accompanying growth of the fungus. Generally, it grew slowly in malt agar and the white mycelial mat turned into creamy patches at maturity. Aerial mycelia were likewise present with a

slight thickening as growth progressed. It was without odor and the reverse side of the plate was unchanged.

Decay Test

The five species of basidiomycetes caused weight losses ranging from 4.9 to 22.3% on ponderosa wood blocks (Table 6). ***Basidio #1*** produced the largest average weight loss (22.3%) among the basidiomycetes tested. Of the species of fungi that were classified as white rotters, ***Polyporus #1*** caused an average weight loss of 17.3%, followed by ***Polyporus #2***, ***Trametes versicolor*** and ***Heterobasidium annosum*** with 13.5%, 9.9% and 4.9% weight losses, respectively.

Variations in the degree of attack were noted with the test fungi although mycelial growth was normally observed covering the surfaces of the blocks. Blocks exposed to ***Heterobasidium annosum*** were covered with mycelia but weight losses were relatively low.

The average percent weight losses caused by basidiomycetous fungi were lower than those caused by ***P. placenta***. The soil block test methodology employed was generally more suited to growth of brown rot fungi. On the other hand, the low average percent weight losses on the blocks exposed to the brown rot fungus, ***Basidio #1***, may

Table 6. Average Percent Weight Losses of *Ponderosa* Pine Blocks Caused by Selected Fungi Isolated from Adults of Golden Metallic Beetles After 12 Weeks of Exposure.[a/]

Wood-decay Fungi	Ave.% Wt.Loss		Isolation Freq.(%)
Basidio #1	22.3	(3.6)	0.4
Polyporus #1	17.3	(1.5)	1.1
Polyporus #2	13.5	(3.2)	0.5
Trametes versicolor	9.9	(1.6)	0.2
Hetererobasidium ann	4.9	(0.5)	0.6
Postia placenta	37.3	(16.6)	-
Control	0.05	(0.1)	-

[a/] Values represent means of 5 replicates. Figures in parenthesis represent standard deviations.

wood are present in or on ***B. aurulenta L.*** adults. Although they occurred at a low frequency, their importance in the colonization or decomposition process cannot be discounted. As such a single spore carried by an insect into a suitable host could develop and eventually produce a fruiting body containing numerous spores. A decay fungus ***Ganoderma applanatum*** has been reported to produce billions of spores daily (Zabel and Morrell, 1992). On the other hand, the development of basidiomycetous structures and white rot on ***Abies amabilis*** occurred two years after the invasion by xylophagous beetles (Schowalter et al., 1992).

The mechanism of transport of basidiomycetous fungi in golden metallic beetles has not been investigated. For this reason, further trials will be required to determine if these propagules are, in fact, transferred during oviposition and if such propagules can initiate deterioration.

Characteristics of Frequently Isolated Imperfect Fungi

The following are brief descriptions of the most frequently isolated Fungi Imperfecti:

Trichoderma.. In culture, mycelium was thin, transparent, septate and branched with clusters of green spores. These fungi are cosmopolitan and are very common

in lumberyards. They are widely distributed worldwide and can cause blue to blue-mottled discoloration of pines including ***P. strobus*** and ***P. ponderosa*** (Kaarik, 1980).

Alternaria. Mycelium was thin and became black at maturity. Spores are septate, 3-4 celled, and borne on conidiophores. This genus contains cosmopolitan fungi that can cause stain in logs and lumber of pine, spruce, and hardwoods. These are common in forest soil, plant materials, wood pulp and slime of paper mills (Domsch et al., 1980) and has been commonly isolated from severely brown rotted wood (Wang and Zabel, 1990). ***Alternaria alternata*** produced Type 2 soft-rot damage in southern pine, American beech, and ponderosa pine blocks (Morrell and Zabel, 1985), but caused only moderate amounts of both Types 1 and 2 cell wall damage in Douglas-fir sapwood (Zabel et al., 1990).

Penicillium. The genus ***Penicillium*** consists of more than 300 species (Raper and Thom, 1949; Pitt, 1979; Ramirez, 1982). Mycelium of isolates from the beetles was thin and septate. Conidiophores were phialidic and produced chains of single-celled spores. Members of this genus are common in soil, decaying pine and Douglas-fir (Wang and Zabel, 1990). Levy (1969) reported that this fungus formed soft-rot cavities in European birch and Scots pine wood. However, Nilsson (1973) claimed that this fungus caused

only a slight cell wall erosion in European birch wood and did not produce any soft-rot cavities.

Morphological Features on the External Parts of Golden Metallic Beetle Associated with Isolated Fungi

Head

On the frontal region of the head, numerous small pits located around the base of each soft, slender and long seta were observed. Similar structures were also noted on the sclerotized chewing mouthparts but the setae were shorter and harder than those found on the head (Fig. 2). Beeson (1917) found similar structures on the head of the platypodid ***Diapus furtivus*** Sampson, which he claimed could carry spores. Likewise, fungal spores were abundant in the female-specific pubescent pronotal depression on four species of ***Pityoborus*** (Furniss et al., 1987) which he termed as mycangial setae.

Thorax

Examination of the locomotory tagma of the golden metallic beetles showed numerous oval invaginations on each side of the thorax (Fig. 3). Fungal spores were observed along the length of the setae and also within the sac-like

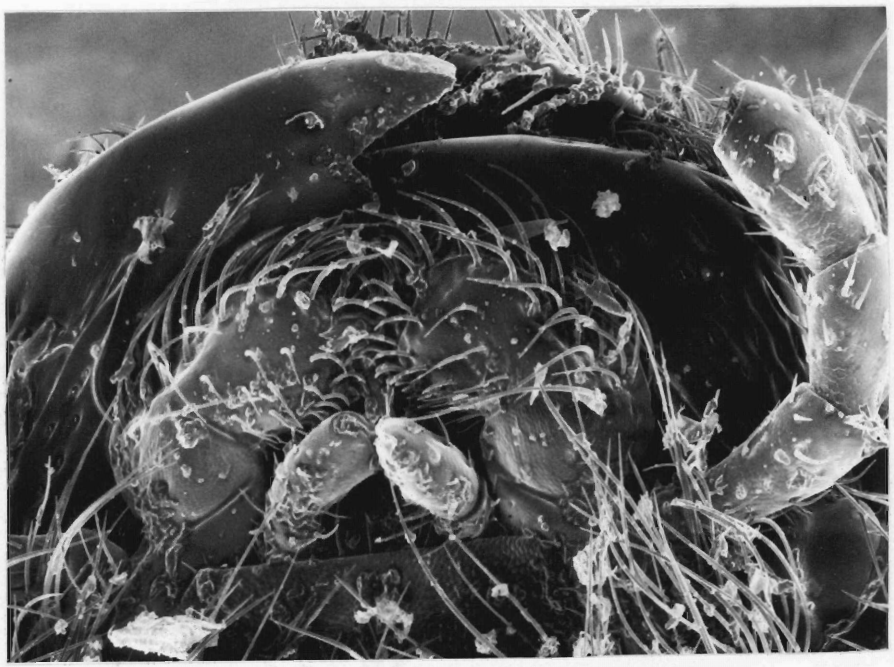

Fig. 2. Ventral View of the Head of a Golden Metallic Beetle Showing Setae on Pairs of Mandibles and Maxillae (100X).

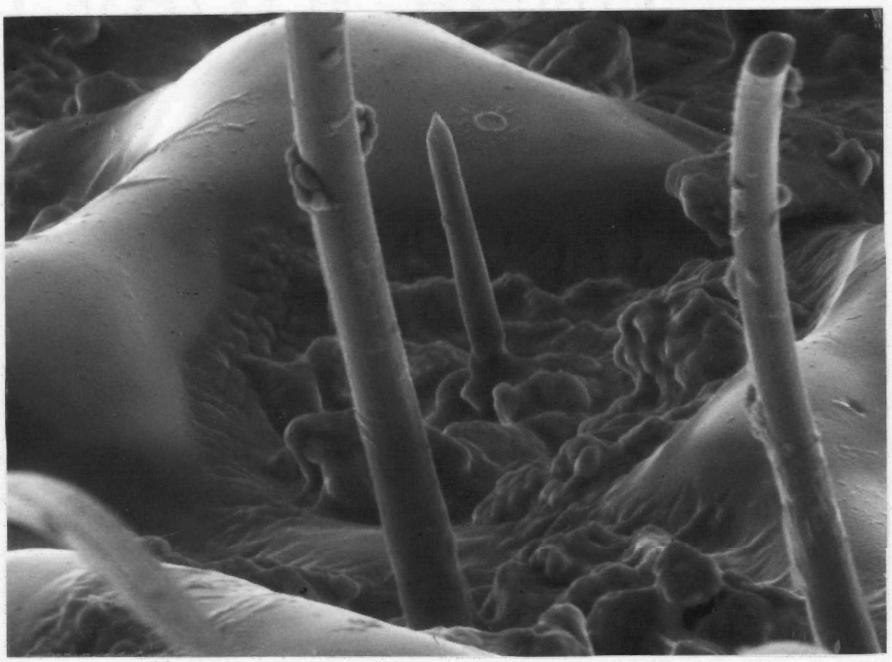

Fig. 3. Dorsal View of the Thorax of a Golden Metallic Beetle Showing Oval Invagination with the Cuticle Containing Wax-like Structure (1300X).

structures or invaginations on the dorsal thorax (Fig. 4). It is probable that the setae comb or brush the fungal mycelia or spores as the beetle crawls on infested logs.

Elytra

Rows of four cup-shaped depressions were found on the surface of each pair of elytra. Each depression contained a seta whose length was the same as the depth of the depression (Fig. 5). A considerable number of fungal isolates were obtained from the elytra (Table 1).

Legs

The legs of the beetle contained numerous pubescent structures which were more or less equidistantly spaced (Fig. 6). These structures were found on all three pairs of legs except that the setae on the tibia where shorter and harder than those in the femur. The setae and repository-like structure around the base were observed to carry fungal spores (Fig. 7). The crawling movement of the beetles on infected logs may brush the fungal masses and result in the gathering of more spores on these locomotory organs as compared to other body parts.

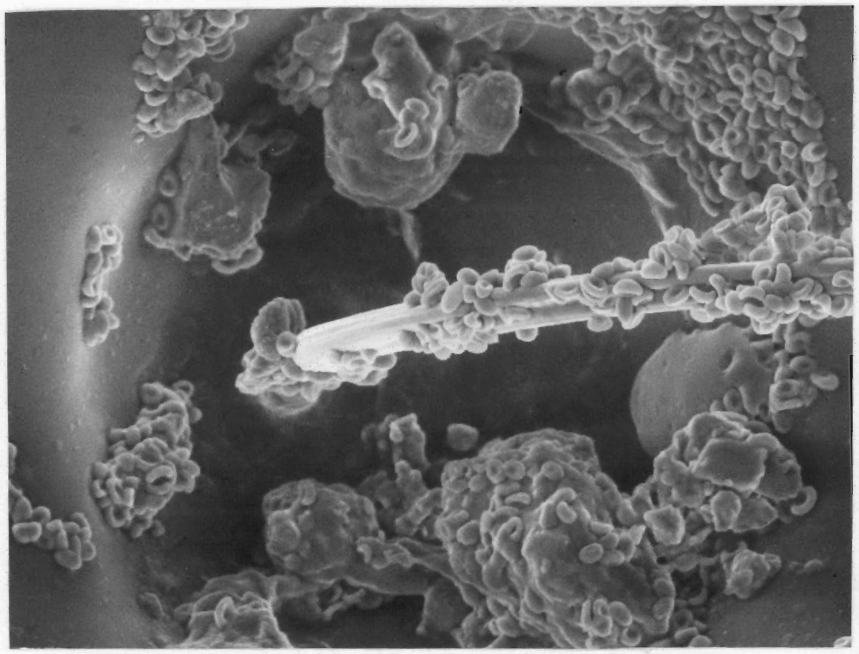

Fig. 4. Cuticular Invagination and a Seta of a Golden Metallic Beetle Showing Numerous Fungal Spores (1300X).

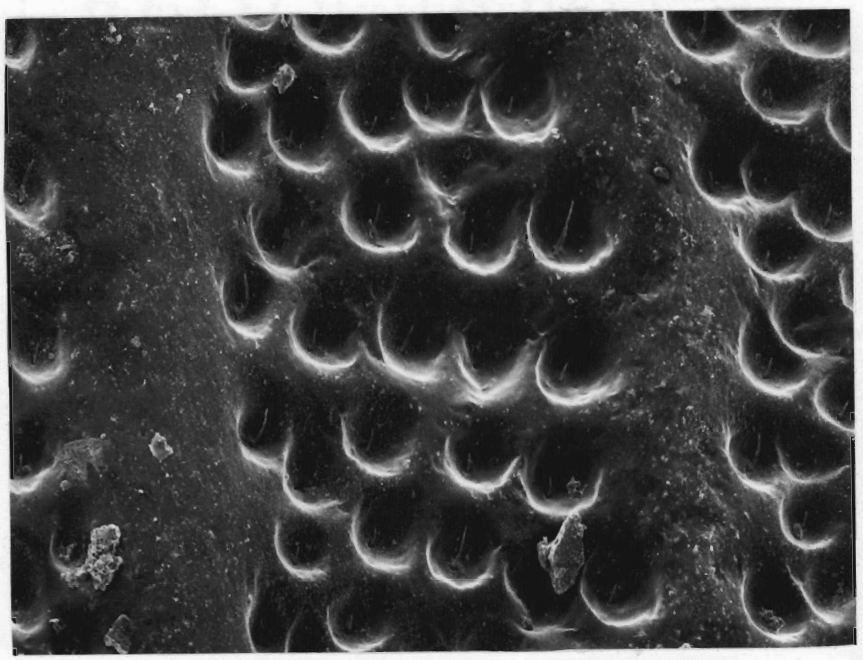

Fig. 5. Cup-Shaped Depression in Rows of Four on the Elytra of a Golden Metallic Beetle Showing Pubescent Areas (120X).

Fig. 6. Pubescent Structures on the Thoracic Legs of a Golden Metallic Beetle (400X).

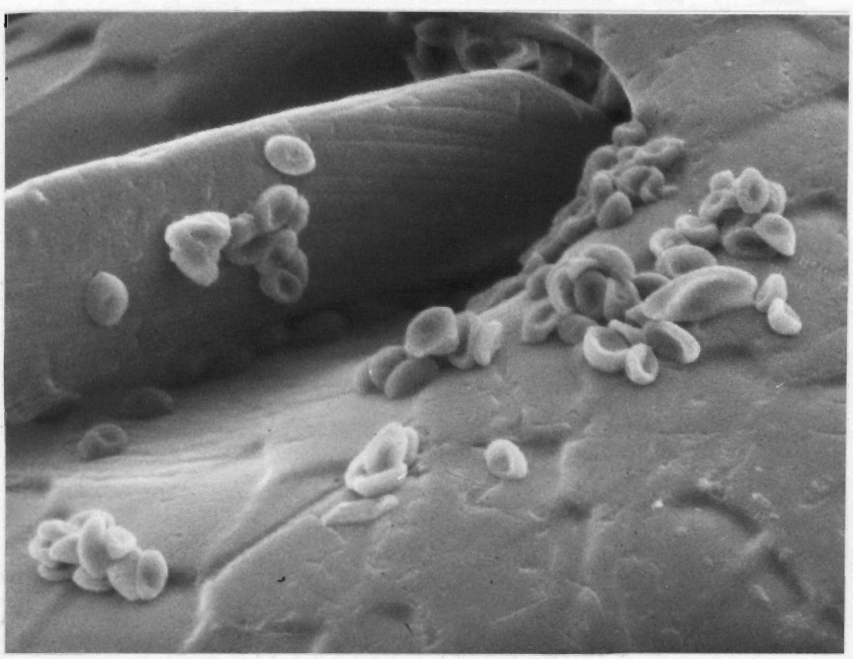

Fig. 7. Highly Magnified Portion of a Seta of a Golden Metallic Beetle with a Repository Structure Containing Fungal Spores (2500X).

The setae and invaginations of the insect cuticle on the above-mentioned body parts of the golden metallic beetle were presumed to be structures that could be implicated in the transport of fungi from infected to freshly fallen logs (Batra, 1963; Livingston and Berryman, 1972; Beaver, 1986; Furniss et al., 1987; Levieux et al., 1991). The minute size of a single spore (from 1 to 200 microns in diameter) makes it adapted for the transport in these mycangial structures. The beetles were presumed to acquire the fungi as they crawled in the older infested piles of logs.

Fungi could also be transported internally by golden metallic beetles as shown by the number of fungi isolated from the gut (Table 5), although the ability to survive in this venue may be sharply reduced. Fungal spores have been known to be ingested by beetles and passed out in the excreta uninjured (Leach, 1940). Examination of the ovipositor of the beetle showed specialized structures protruding on each side of the posterior end of the female genitalia. The function of the specialized structure was unknown but it may facilitate the dissemination of fungi during oviposition between cracks and crevices in logs. The inclusion of fungal mycelium or spores along with eggs provides an ideal opportunity for introduction of agents of

deterioration which could condition the wood and concentrate nutrients for the developing larvae.

CONCLUSIONS

Adult golden metallic beetles were characterized by the presence of a wide array of associated fungi capable of both conditioning the wood and producing total wood degradation. Spores and hyphal fragments were noted at a variety of locations on the surfaces of the beetles, suggesting that spore acquisition can easily occur as beetles leave older, infested materials in search of raw host logs. The delineation of the importance of these fungi in successful beetle establishment and the concurrent role of the beetles in establishing new fungal infestations will require more detailed study.

LITERATURE CITED

Ainsworth, G.C. 1966. A general purpose classification for fungi. Bibl. Syst. Mycol. 1:1-4.

American Society for Testing and Materials. 1986. Method of Accelerated Laboratory Test of Natural Decay Resistance of Woods. ASTM D2017-81. Ann. ASTM Standards, Section 4, Volume 04.09. Philadelphia. PA.

Bajan, C., K. Kmitowa, M. Wojciechowska, and A. Fedorko. 1977. The Effect of Entomopathogenic Microorganisms Introduced into the soil on the Development of Successive Generations of the Colorado Beetle. Pol. Ecol. Stud. 3:157-165.

Baker, J.M. 1963. Ambrosia beetles and their fungi, with particular reference to ***Platypus cylindrus*** Fab. Symp. Soc. Gen. Microbiol. 13:232-265.

Barras, S.J. and T. Perry. 1971. Gland cells and fungi associated with prothoracic mycangium of ***Dendroctonus adjunctus*** (Col., Scol.) Ann. Ento. Soc. Am. 64:123-126.

Barras, S.J. and T. Perry. 1972. Fungal symbionts in the prothoracic mycangium of ***Dendroctonus frontalis*** (Coleoptera: Scolytidae). z. Angew. Entomol. 71:95-104.

Barras, S.J. and J.J. Taylor. 1973. Varietal ***Ceratocystis minor*** identified from mycangium of ***Dendroctonus frontalis***. Mycophathol. Mycol. Appl. 50:293-305.

Batra, L.R. 1963. Ecology of ambrosia fungi and their dissemination by beetles. Trans. Kans. Acad. Sci. 66:213- 236.

Batra, S.W. and L.R. Batra. 1967. The fungus gardens of insects. Sci. Am. 217(5):112-120.

Beaver, R.A. 1986. The taxonomy, mycangia and biology of ***Hypothenemus curtipennis*** (Schedl), the first known cryphaline ambrosia beetle (Coleoptera: Scolytidae). Entomol. Scand. 17:131-135.

Beeson, C.I. 1917. The life-history of ***Diapus furtivus*** Sampson Indian For. Res. 6:1-29.

Berryman, A.A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. Biol. Sci. 22:598-602.

Berryman, A.A. 1989. Adaptive pathways in scolytid-fungus associations. *In* "Insect-fungus Interactions" (N. Wilding, N.M. Collins, P.M. Hammond and J.F. Webber, eds). Academic Press, New York. pp 145-159.

Boyce, J.S. 1961. "Forest Pathology". 3rd Ed. McGraw-Hill, N.Y. 572 pp.

Brand, J.M. and S.J. Barras. 1977. The major volatile constituents of a basidiomycete associated with the southern pine beetle. Lloydia. 40:318-399.

Brand, J.M., J.W. Bracke, L.N. Britton, A.J. Markovetz, and S.J. Barras. 1976. Bark beetle pheromones: production of verbenone by a mycangial fungus of ***Dendroctonus frontalis***. J. Chem. Ecol. 2:195-199.

Bridges, J.R. and J.C. Master. 1983. Role of two phoretic mites in transmission of blue-stain fungi ***Ceratocystis minor***. Ecol. Ent. 8:9-12.

Burke, 1918. Biological notes on some flat-headed woodborers of the genus ***Buprestis***. J. Econ. Ent. 11:334-338).

Carpenter, S.E. M.E. Harmon, E.R. Ingham, R.G. Kelsey, J.D. Lattin and T.D. Schowalter. 1988. Early Pattern of Heterotroph Activity in Conifer Logs. ***In*** "Fungi and Ecological Disturbance. R. Wathing, A. Lyong and L. Boddy (eds.) Proc. Royal Soc. Of Edenburgh 94B. pp 33-43.

Chamberlin, W.J. 1924. Notes on the Buprestidae of Oregon with descriptions of new species. J.N.Y. Ent. Soc. 32: 186-195.

Chamberlin, W.J. 1947. The Golden Buprestidae: A household pest. OSU, Agr. Exp. Sta., Circ. 417, Corvallis, OR.

Chamberlin, W.J. 1949. Insects affecting forest products and other materials. Published by OSU Cooperative Extension Service. Corvallis, OR.

Chapman, A.D. and T.C. Scheffer. 1940. Effect of blue-stain on specific gravity and strength of southern pine. J. Agr. Research. 61(2):125-134.

Cobb, Jr., F.W. 1988. ***Leptographium wageneri***, cause of blackroot disease: a review of its discovery, occurrence, and biology with emphasis on pinyon and ponderosa pine. *In* "***Leptographium*** Root Diseases on Conifers" (T.C. Harrington and F.W. Cobb, Jr. eds.) pp 41-62. American Phytopathological Society Press, St. Paul, MN.

Cody, R.P. and J.K. Smith. 1991. Applied Statistics and the SAS® Programming Language. 3rd Edition. Prentice Hall, Englewood Cliffs, N.J. 398.pp.

Craighead, F.C. 1928. Interrelation of tree-killing bark beetles (**Dendroctonus**) and blue-stain. Jour. Forestry 26:886-887.

Crowson, R.A. 1984. The associations of Coleoptera with Ascomycetes. *In* "Fungus-Insect Relationships: Perspectives in Ecology and Evolution". (Q.D. Wheeler and M. Blackwell, eds). Pp 256-258. Columbia Univ. Press, New York.

Davidson, R.W., W.A. Campbell and D.J. Balisdell. 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. 57:683-695.

Doane, R.W. and O.J. Gilliland. 1929. Three Californian ambrosia beetles. J. Econ. Entomol. 22:915-921.

Domsch, K.H., W. Gams, and T.H. Anderson. 1980. Compendium of Soil Fungi. Vols I & II. Academic Press, N.Y. 859&405 p, respectively.

Dowding, P. 1969. The dispersal and survival of spores of fungi causing blue-stain in pine. Trans. Br. Mycol. Soc. 52:125- 137.

Edmonds, R.L. and A. Eglitis. 1989. The role of the Douglas-fir beetle and wood borers in the decomposition and nutrient release from Douglas-fir logs. Can. J. For. Res. 19:853- 859.

Ellis, M.B. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew, England. 586 p.

Ellis, M.B. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute. Kew, England. 608 p.

Every, R.W. and J.A. Rudinsky. 1975. The Golden Buprestid: A wood boring beetle. OSU Extension Service. Extension Circular 713.

Farguess, J. J.C. Delmas, and R.A. Lebrun. 1994. Leaf Consumption by Larvae of the Colorado Potato Beetle (Coleoptera: Chrysomelidae) Infected with the Entomopathogen, ***Beauveria bassiana***. Journal of Economic Entomology. 87(1): 67-71.

Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rossman. 1989. fungi on Plants and Plant Products in the United States. APS Press, St. Paul, MN. 1252 p.

Forbes, S.A. 1884. The tarnished plant-bug. Farmers' Review. February.

Francke-Grosmann, H. 1956. Hautdrusen als Trager der Pilzesymbiose bei ambrosiakafern. Z. Morphol. Oekol. Tiere 45:275-308

Francke-Grosmann, H. 1963. Some new aspects in forest entomology. Ann. Rev. Entomol. 8:415-438.

Francke-Grosman, H. 1967. Ectosymbiosis in wood-inhabiting insects. In "Symbiosis" (S.M. Henry, ed.), Vol. 2, pp 141-205. Academic Press, New York, N. Y.

Franz, E. 1936. Ein nord-amerikanischer Kafer in einer Frankfurter Aurichte. Natur U. Volk. 66:370.

Furniss, M.M., J.Y. Woo, M.A. Deyrup and T.H. Atkinson. 1987. Prothoracic Mycangium on Pine-infesting ***Pityoborus*** spp. (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 80:692-696.

Garnett, R.T. 1918. Notes on the genus ***Buprestis*** Linne, in California. Ann. Ent. Soc. Amer. 11:90-92.

Gibbs, J.G. and D.W. French. 1980. The transmission of oak wilt. USDA Forest Service Research Paper: NC 185. pp 1-17.

Goheen, D.J., and E.M. Hansen. 1993. Effects of pathogens and bark beetles on forests. In "Beetle Pathogen Interactions in Conifer Forests" (T.D. Schowalter and G.M. Filip eds) pp. 175- 196. Academic Press, London.

Graham, K. 1967. Fungal-insect mutualism in trees and timber. Annual Rev. Ent. 12:105-126.

Happ, G.M., C. Happ and S.J. Barras. 1971. Fine structure of the prothoracic mycangium, a chamber for the culture of symbiotic fungi in the southern pine beetle, ***Dendroctonus frontalis***. Tissue and Cell 3(2):295-308.

Hartig, R. 1947. Wichtige Krankheiten der Waldbaume. Berlin. Verlag Julius Springer.

Hatch, M.H. 1971. The Beetle of the Pacific Northwest. Univ. of Washington Press, Seattle. Vol.16. 662 pp.

Helfer, J.R. 1941. A revision of the genus ***Buprestis*** of N. America north of Mexico. Ent. Amer. 21(3):123-199.

Huguenin, J.C. 1915. An observation on a buprestid. Ent. News 26:364-365.

Ingold, C.T. 1978. Dispersal of micro-organisms. In "Plant Disease Epidemiology" (P.R. Scott and A. Bainbridege, eds), pp. 11-22 Blackwell Scientific, Oxford. UK.

Kaarik, A. 1980. Fungi causing sap stain in wood. The Swedish University of Agricultural Sciences. Report Nr R 114. 112 p.

Kok, L.T., D.M. Norris and H.M. Chu. 1970. Sterol metabolism as a basis for a mutualistic symbiosis. Nature (London) 225:661-662.

Kok, L.T. 1979. Lipids of ambrosia fungi in the life of mutualistic beetles. In "Insect-Fungus Symbiosis". (L.R. Batra, ed.) pp. 33-52. Halsted Press, Chechester, Sussex. UK.

Leach, J.G. 1940. Insect transmission of plant diseases. 1st Ed. Mc. Graw Hill Book Co. Inc. N.Y. & London. 615 pp.

Lekander, B., B. Bejer-Peterson, E. Kangas, and A. Bakke. 1977. The distribution of bark beetles in Nordic countries. Acta Entomol. Fenn. 32:1-37.

Levieux, J., P. Cassier, D. Guillaumin and A. Roques. 1991. Pathogenic Fungi by the European Bark Beetle, ***Ips sexdentatus*** Boerner: Ultrastructure of a Mycangium. Can. Ent. 123:245-254.

Liese, W. and R. Schmid. 1964. Uber das Wachstum von Blauepilzen durch verholtze Zellwande. Phytophatol. Z. 51:385-393.

Linsley, E.G. 1943. Delayed emergence of ***B. aurulenta*** from structural timbers. J. Econ. Ent. 36(2)348-349.

Livingston, R.L. and A.A. Beryman. 1972. Fungus transport structures in the fir engraver, ***Scolytus vertralis*** (Coleoptera: Scolytidae). Insecta Matsumura 7.

Morrell, J.J. and R.A. Zabel. 1985. Wood strength and weigh losses caused by soft-rot fungi isolated from treated southern pine utility poles. Wood Fibers Sci. 17: 132-143.

_____. **1987.** Partial delignification of wood: its effect on the action of soft-rot fungi isolated from preservative-treated southern pines. Mater. Org. 22:215-224.

Neger, F.W. 1911. Zur "Ubertragung des Ambrosiapilzes von ***Xyleborus*** dispar. Naturwiss. Z. Forst landwisrtsch. 223-225.

Nelson, R.M. and J.A. Beal. 1929. Experiments with bluestain fungi in southern pines. Phytopath. 19:1101-1106.

Nobles, M.K. 1948. Studies in Forest Pathology VI. Identification of cultures of wood-rotting fungi. Division of Botany and Plant Pathology Science Service. Canadian Journal of Research C, 26:281-431. Published by authority of the Right Hon. James G. Gardiner, Minister of Agriculture, Ottawa, Canada.

Norris, D.M. and J.K. Baker. 1967. Symbiosis: effects of a mutualistic fungus upon the growth and reproduction of ***Xyleborus ferrugineus***. Science 156:1120-1122.

Norris, D.M. 1979. The mutualistic fungi of xyleborine beetles. In "Insect-Fungus Symbiosis". (L.R. Batra, ed.). pp. 53-63 Halsted Press, Chichester, Sussex,.UK

Nunberg, M. 1951. Contribution to the knowledge of prothoracic glands of Scolytidae and Platypodidae (Coleoptera). Ann. Mus. Zool. Pol. 14:261-265.

Purcell, A.H. 1982a. Evolution of the insect vector relationship. In "Phytopathogenetic Prokaryotes, vol. 1" (M.S. Mount and G.H. Lacey Ed.) pp 121-156. Academic Press, New York.

Ramsey, F. and D. Schafer. 1994. The Statistical Sleuth: A Second Course in Statistical Data Analysis. OSU Book Store, Inc. Book Store Printing. pp 102-110.

Reid, R.W., H.S. Witney and J.A. Watson. 1967. Reactions of lodgepole pine to attack by ***Dendroctonus ponderosae*** Hopkins and blue-stain fungi. Can. J. Bot. 46:1115-1126.

Rumbold, C.T. 1931. Two blue-staining fungi associated with bark beetle infestation of pines. J. Agric. Res. 43(10:847-873.

Scheffer, T.C., J.J. Morrell, and M.A. Newbill. 1988. Laboratory Evaluation of Potential Replacements for Pentachlorophenol in sprays to Control Sapwood Decay in Western Redcedar Poles. Holzforschung 42:185-189.

Schowalter, T.D., B.A. Caldwell, S.E. Carpenter, R.D. Griffith, M.E. Harmon, E.R. Ingham, R.G. Kelsey, J.D.Lattin and A.R. Moldenke. 1992. Decomposition of Fallen Trees: Effects of Initial Conditions and Heteroph Colonization Rates. ***In*** "Tropical Ecosystems Ecology and Management. Singh, K.P. and J.S. Singh Eds. Wile Eastern Limited. New Delhi. pp 373-383.

Smith, D.N. 1962. A note on the longevity and behavior of adult golden buprestids, ***B. aurulenta*** **L.** (Coleoptera: Buprestidae) under artificial conditions. The Canadian Entomologists. June 1962 pp 590-591.

Spencer, G.C. 1930. Insects emerging from prepared lumber in building. Proc. Ent. Soc. British Columbia. 1930:6-10.

Stephen, F.M., C.W. Berisford, D.L. Dahlsten, P. Fenn and J.C. Moser. 1993. Invertebrate and Microbial Associates. ***In*** "Beetle-Pathogen Interactions in Conifer Forests". Schowalter, T.D. and G.M. Filip (Ed.). 1993. Academic Press. San Diego, CA. Ch. 7 pp 129-153.

Stewart, V.B. 1913. The importance of the tarnished plant bug in the dissemination of fire blight in nursery stock. Phytopath. 3: 273-276.

Swift, M.J. and L. Boddy. 1984. Animal-microbial interactions in wood decomposition. In "Invertebrate-Microbial Interactions" (J.M. Anderson, A.D.M. Rayner and D.W.H. Walton, eds) pp 89-131. Cambridge Univ. Press, Cambridge, UK.

Van Dyke, E.C. 1939. An Ancient Beetle. Pan-Pacific Ent. 15:154.

Von Schrenk, H. 1903. The "Bluing" and the "Red Rot" of the western yellow pine, with special reference to the Black Hills Forest Reserve. U.S. Dept. Agr. Bur. Plant Indus. Bull. 36.

Waite, M.B. 1891. Results from recent investigations in pear blight. Bot. Gaz. 16: 259.

Wang, C.J.K. and R.A. Zabel. 1990. Identification Manual for Fungi from Utility Poles in the eastern United States. Allen Press Inc. Lawrence, Kansas. 356 pp.

Webber, J.F. and J.N. Gibbs. 1989. Insect dissemination of fungal pathogens of trees. In "Insect-Fungus Interactions" 14th Symposium of the British Royal Entomological Society (N. Wilding, N.M. Collins, P.M. Hammond and J.F. Webber, eds). Academic Press, London. UK. pp 161-193.

Whitney, H. S. 1982. Relationships between bark beetles and symbiotic organisms. In "Bark Beetles in North American conifers" (J.B. and K.B. Sturgeon, eds), pp 183-211. Univ. of Texas Press, Austin, TX.

Wilding, N., N.M. Collins, P.M. Hammond and J.F. Webber (Ed.). 1989. Insect-fungus interactions. 14th Symposium of the Royal Ent. Soc. of London. Academic Press. San Diego CA. 344 pp.

Witcosky, J.J., T.D. Schowalter, and E.M. Hansen. 1986. ***Hylastes*** and ***Steremnius carinatus*** (Coleoptera: Curculionidae) as vectors of black stain root diseases of Douglas-fir. Environ. Entomol. 15:1090-1095.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Col., Scolytidae), a taxonomic monograph, Great Basin Nat. Mem. 6:1-1359.

Zabel, R.A., C.J.K. Wang and S. Anagnost. 1990. Soft-rot capability of the major microfungi isolated from Douglas-fir poles in the Northeast. In: Identification for fungi from utility poles in the eastern United States (Wang, C.J.K. and R.A. Zabel eds.) Am. Type Culture Collection. Allen Press, Inc.. Lawrence, Kansas. 356p.

Zabel, R.A. and Morrell. 1992. Wood Microbiology: Decay and Its Prevention. Academic Press, Inc. San Diego, CA. 476 pp.

APPENDICES

Appendix 1. Criteria Used for the Evaluation of the Growth Response of the Test Fungi Grown on Gallic and Tannic Acid Culture Media (Davidson et al., 1938).

\- negative, no brown discoloration of agar observed.

\+ positive, diffusion zone light to dark brown, formed under inoculum at center of mat and visible from under side of dish. Also included in this category are cases when no mat develops and only a faint brown discoloration is produced directly under the inoculum.

++ positive, diffusion zone light to dark brown, formed under most of mat but not extending to margin. Visible from under side only.

+++ positive, diffusion zone light to dark brown, extending a short distance beyond the margin of the mat and visible from upper side. This rating is so used when the mat covers the entire agar surface and the agar beneath it is completely brown. This situation may arise at 2 weeks in some ***Peniophora*** species.

++++ positive, diffusion zone light to dark brown, opaque or not extending considerably beyond margin of fungus mat (or inoculum plug no mat develops).

+++++ positive, diffusion zone very intense, dark brown, opaque, forming a wide corona about mat (or inoculum plug when no mat develops).

Appendix 2. Key to Grown Groups of Basidiomycetes on Gallic and Tannic Acid Culture Media (Davidson et al., 1938).

1. Negative: Gallic = Tannic
1a. Negative: Gallic < Tannic

2. Negative: Gallic good, > Tannic

3. Negative: Gallic good, Tannic 0 to trace

4. Positive: Gallic 0 to trace
Tannic 0 to trace

4a. Positive: Gallic 0 to 25 mm,
Tannic 0 to trace

5. Positive: Gallic 0 to trace
Tannic 0 to 25, strong reactors

6. Positive: Gallic 0 to trace
Tannic 25-50 mm

7. Positive: Gallic = Tannic

8. Positive: Gallic fair, Tannic good

9. Positive: Gallic good, tannic 0 to trace, weak reactors

10. Gallic negative, good
Tannic positive, good

10a. Gallic negative 0-25 mm
Tannic positive 0-25 mm

11. Gallic positive 0 to trace
Tannic negative 0

APPENDIX TABLES

Appendix Table 1. Wilcoxon Rank Sum Test for Basidiomycetes Classified by Variable Sex from NPAR1WAY Procedure Using the SAS Software.

SEX	N	SUM OF SCORES	EXPECTED Under Ho:	STD. DEV. Under Ho:	MEAN SCORE
FEMALE	59	2330.00	2389.5000	65.2360	39.4915
MALE	21	910.00	850.5000	65.2360	43.3333

Z = 0.904409 Prob > /Z/ = 0.3658

T - test Approx. Significance = 0.3685

Appendix Table 2. Wilcoxon Rank Sum Test for Fungi Imperfecti Classified by Variable Sex from NPAR1WAY Procedure Using the SAS Software.

SEX	N	SUM OF SCORES	EXPECTED Under Ho:	STD. DEV. Under Ho:	MEAN SCORE
FEMALE	59	2333.50	2389.50	91.2544	39.5508
MALE	21	906.50	850.50	91.2544	43.1667

Z = 0.608190 Prob > /Z/ = 0.5431

T - test Approx. Significance = 0.5448

Appendix Table 3. Wilcoxon Rank Sum Test for Ascomycetes Classified by Variable Sex from NPAR1WAY Procedure Using the SAS Software.

SEX	N	SUM OF SCORES	EXPECTED Under Ho:	STD. DEV. Under Ho:	MEAN SCORE
FEMALE	59	2327.50	2389.50	71.7593	39.4492
MALE	21	912.50	850.50	71.7593	43.4524

Z = 0.857032 Prob > /Z/ = 0.3914

T - test Approx. Significance = 0.3940