AN ABSTRACT OF THE THESIS OF

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Title: Toxicity of Methyl Bromide to Fungi Inhabiting Dahurian Larch Wood

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Concerns over the possibility of exotic pest introductions from eastern Russia to the West Coast of the United States due to proposed log imports raises the question of the effectiveness of possible mitigation measures. Toxicity of methyl bromide to representative pathogenic fungi was tested by exposing *Armillaria ostoyae, Heterobasidion annosum, Lachnellula wilkommii* and *Leptographium wageneri* grown in one centimeter media-amended Dahurian larch (*Larix gmelinii*) wood cubes to initial concentrations of 0, 79 or 237 mg/l of methyl bromide for 8, 24 or 72 hours. All fumigations were performed at 18° C in sealed glass jars. Leakage of fumigant from the jars and sorption into the cubes was closely monitored. After fumigation, cubes were quartered and plated on malt agar. The percentage of cube quarter sections with no visible growth after two weeks was used as a bioassay of fumigation efficacy. Survival was noted at concentration time values (CxT) as high as 1267 mgh/l for *Armillaria ostoyae*, 3006 mgh/l for *Heterobasidion annosum*, 1230 mgh/l for *Lachnellula wilkommii* and 4748 mgh/l for *Leptographium wageneri*. Although these values may be reached in the thin sapwood of Dahurian larch logs, they will probably not be approached in the heartwood of these logs using conventional tent fumigation techniques.

Toxicity of Methyl Bromide to Fungi Inhabiting Dahurian Larch Wood

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Ronald G. Rhatigan

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Major Professor, representing Forest Science

Chair of Department of Forest Science

Dean of Graduate School

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

Ronald G. Rhatigan, Author

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I INTRODUCTION

Many lumber mills from the western United States are exploring different sources of log supply. One potential source of supply that has interested several timber companies is eastern Siberia and the Russian Far East. These areas contain immense timber reserves. Tseplyaev (1965) reported that the forested area in these two regions of Russia totaled 268,706,000 hectares. Most of this forest (60.3%) is dominated by larch (*Larix* spp.). Total larch wood reserves in these two areas are reported to be 27.8 billion cubic meters. The USDA, Forest Service "Pest Risk Assessment of the Importation of Larch from Siberia and the Soviet Far East" (1991), noted that the genus *Larix* attains its greatest global concentration and diversity in Eastern Siberia and the Russian Far East. There is no clear consensus of the number of larch species or varieties in this area. Most Western sources list only a few: Farjon (1990) listed one species with four varieties, Rushforth (1987) listed two species and two varieties, and Silba (1986) listed one species with three varieties. The Russian source used by the previously cited Forest Service publication (Solokov, 1977) listed nine. Since it is the reference chosen by the USDA, Forest Service, it will be accepted for the purposes of this study.

The USDA, Forest Service (1991) consider Dahurian larch (*Larix gmelini* [Rupr.] = *Larix dahurica* Turcz. et Trautv.) and Amur larch (*Larix amurensis* B. Kolesn) to be the only commercially important larch timber species of eastern

Siberia and the Russian Far East; Dahurian Larch will likely make up the bulk of the logs to be imported. The other species have limited ranges and economic importance, although small volumes may be included in log shipments. Larch timber is similar to that of Douglas-fir (*Pseudotsuga menzesii* Mirb. [Franco]), and it would likely be used as a replacement for this species. However, an area with such a concentration and diversity of hosts could very likely contain a similar abundance of coevolved injurious insects and pathogens. Therefore, before Russian larch can be imported into the United States, methods must be identified which destroy the many pest species on and inside the logs which might pose a threat to the forests of the West Coast.

For example, the previously cited USDA, Forest Service pest risk assessment estimated that the likely economic costs of introducing a new strain of annosus root disease caused by *Heterobasidion annosum* [Fr.] Bref. into the western United States from these areas could reach as high as $343 million. Estimated costs associated with the introduction of the Asian gypsy moth (*Lymantria dispar* L.) are many times greater. Debarking logs will eliminate most of the insect species and many of the superficial fungal infestations. It will not, however, affect the many pest species present deeper inside the logs, most of which are fungi. Before specific mitigation measures can be approved, they must be proven capable of destroying all potentially harmful organisms to the center of each imported log.

II LITERATURE REVIEW

A. Risks of Forest Pest Introductions

North America possesses vast, productive forest lands that are vulnerable to degradation from imported forest pests. Chestnut blight, caused by *Chryphonectria parisitica* (Murr.) Barr is the most destructive plant disease ever recorded. It reduced the American chestnut (*Castanea dentata* [Marsh.] Borkh.) from the most economically important hardwood species of the eastern United States to mere stump sprouts (USDA, Forest Service, 1991). The impact of Dutch elm disease, caused by *Ophiostoma ulmi* Busim. and its insect vectors on North America's urban forests has also been immense. Approximately 45 million urban elms were killed by the disease between 1930 and 1977. The monetary loss considering removal costs and lowered property values was estimated at $430 per tree (Stipes and Campana, 1981). White pine blister rust, caused by *Cronartium ribicola* Fisher results in annual growth losses and mortality of 5.7 million cubic meters of the very valuable white pine species (Skilling, 1975); this is equivalent to 3.2% of the total growth impact on the commercial forests of the U.S. (Partridge et al., 1977). This disease has also made the management of these timber types unprofitable over much of the white pine forest lands. As early as 1898 Dr. Carl A. Schenk (America's first Forestry professor) warned against the danger of importing white pine nursery stock from Europe due to the prevalence of blister rust there. His warnings went unheeded and his prediction of disaster was fulfilled (Benedict, 1981).

A list of exotic pests known or strongly suspected to be introduced on wood products and associated economic effects has been compiled for this report to illustrate the seriousness of the threat (Appendix A). The list is arranged taxonomically according to insect pest or vector. This summary reveals some interesting trends. All pathogens known to be introduced on logs or wood products are, as the Dutch elm disease fungus, insect vectored. They may, as with the Dutch elm disease fungus, be carried by native and/or exotic insects. The economic and environmental effects are often minor or cryptic but occasionally, major and devastating.

Certainly many destructive forest pathogens such as chestnut blight, white pine blister rust, and Port-Orford-cedar root disease caused by *Phytophthora lateralis* Tucker and Milbrath have been introduced to areas where they are not native. In these cases and many others, infested nursery stock was the suspected or known mode of entry. McCubbin (1954) estimates that 90% of the agricultural pests which have come to the United States from abroad have been imported along with plants, nursery stock or seed.

In the case of some forest pathogens the mode of entry is much less certain. *Phytophthora cinnamomi* Rands is a pathogen of over 900 recorded hosts that was probably introduced to North America and other regions from southeast Asia via infested soil, not necessarily soil associated with nursery stock (Zentmyer, 1980). The exotic status of other forest diseases such as the cypress canker, caused by *Seiridium cardinale* (Wagener) Sutton & Gibson in California is debatable and may never be known (Wagener, 1939). Some other forest pathogens such as Eurasian strains of *Heterobasidion annosum* and *Phellinus pini* (Thore: Fr.) A. Ames may have already been introduced via wood products. The

non-aggressive nature of certain strains, or their similarity to native diseases would have rendered them impossible to differentiate without recently developed immunological and genetic techniques.

However, this is probably not the case; North American trade in wood products has largely been export oriented. Imports have overwhelmingly come from tropical areas that have very different environments with very different pest-host complexes than temperate regions. The importation of large volumes of unprocessed wood products from temperate regions to North America is a relatively recent development.

C.E. Yarwood in his "History of Plant Pathogen Introductions" (1983) documented the hypothesis previously advanced by many other plant pathologists; most pathogens of agricultural crops in the U.S. have been or will be introduced from abroad. There is ample evidence to support his hypothesis using the example of the larch forests of North America. Moreover, in this situation the hypothesis can be extended to include damaging forest insects as well. The needles of this genus are particularly susceptible; many insects and fungal pathogens attack the deciduous foliage. Also, it should be noted that many pests of larch are pests or potential pests of Douglas-fir.

B. Larches of North America

There are three species of larch native to North America: western larch (*Larix occidentalis* Nutt.) is the largest of the world's larches. It occurs only in the Upper Columbia River Basin, where it dominates almost 800,000 hectares of forest land (USDA, Forest Service, 1991).

Tamarack or eastern larch (*L. larcina* [Du Roi] K. Koch) is a boreal species that occurs from the northeastern and Lake States throughout much of Canada. A disjunct population occurs in interior Alaska. Alpine larch (*L. lyallii* Parl.) grows in isolated locations near the timberline of the northern Cascades and northern Rocky Mountains. Non-native larches are also grown in the northeastern U.S.; their rapid early growth and tolerance of infertile soils makes them suitable for pulp, saw timber (Robbins, 1985), and reclamation of degraded farmlands and strip-mines, (Drooz et al., 1985). The primary exotic species used are: *L. decidua* Miller, European larch; *L. kaempferi* (Lambert) Carriere, Japanese larch; and their hybrid *L. x marschlinsii* Coaz.

1. Insect Pests

The larch sawfly (*Pristiphora erichsonii* [Hartig]) is the most destructive insect enemy of larch in North America (Ives, 1976 from Drooz et al., 1985). It is believed to have been introduced due to its relatively late first North American recording in 1880 in Massachusetts (Drooz, 1975 from Drooz et al., 1985), and its lack of native parasites. It is particularly destructive to eastern larch in the Lake States. It also causes growth losses in western larch by repeated defoliation, but has not caused extensive mortality of healthy stands (Furniss and Carolin., 1977). Some measure of control has been obtained through the release of imported parasites.

The larch casebearer (*Coleophora laricella* [Hbn.}) is the most destructive insect enemy of western larch. It was introduced, presumably on planting stock of European larch.

It was first found in North America in 1886 by Hagen in Massachusetts and quickly spread throughout the range of eastern larch in North America where it causes extensive mortality. In 1957 it was found in the range of western larch which was at that time a relatively pest-free species. Over the next few years the build up of casebearer populations resulted in serious growth loss throughout much of the range of western larch. Since the middle 1970's introduced parasites have become established and provided an acceptable level of control (Denton, 1979; Ryan et al., 1987).

In contrast, the native insect pests of larch are relatively benign. The principle native insect pests of eastern and western larch are the spruce budworm, (*Choristoneura fumiferana* [Clemens]) and the western spruce budworm (*Choristoneura occidentalis* [Freeman]), respectively. The spruce budworm can usually only damage eastern larch during an epidemic; at endemic levels it normally feeds on *Abies* and *Picea* (Johnson, 1990). The western spruce budworm is better able to exist on larch foliage at endemic levels. The most serious damage it does to larch is severing the terminal leader which negatively affects both the growth and form (Schmidt and Shearer, 1990).

The larch bud moth, *Zeiraphera improbana* (Walker) occasionally reaches epidemic levels. Several other native, foliage feeding insects and a few stem feeding beetles attack larch, but they rarely cause major damage to healthy larch (Johnson, 1990; Schmidt and Shearer, 1990).

2. Pathogens

The North American native, larch dwarfmistletoe (*Arceuthobium larcis* [Piper] St. John) causes the most damage of any pathogen of western larch. It can infect seedlings as young as three years old and continue throughout the life of the tree. Detrimental effects include: height and diameter growth reduction, top killing, reduced seed viability, lowered tree resistance to insects and other diseases, wood defects and mortality (Schmidt and Shearer, 1990). Eastern dwarfmistletoe (*Arceuthobium pusillium* Pk.) occasionally causes stem diseases in eastern larch, but only when it grows in mixtures with black spruce (*Picea mariana* [Mill.] B.S.P.), the primary host (Johnson, 1990).

Of the other non-fungal pathogens, Hepting (1971) notes that nematodes have been isolated from the roots of eastern and European larch, but no damage has been ascribed to them. Native wood inhabiting nematodes of the genus *Bursaphelenchus* are not considered serious pests of native conifers in the U.S. However, exotic species of this genus are considered very serious threats that will likely be present in imported larch logs and easily vectored to suitable hosts by native or exotic cerambycids of the genus *Monochamus* (USDA, Forest Service, 1991).

Larch canker (*Lachnellula wilkommii* (Hart.) Den. is the most destructive disease of European larch in Europe (Robbins, 1985). The disease was introduced to the U.S. in 1907 on European and Japanese larch seedlings sent from England to Massachusetts (Spaulding and Siggers, 1927). After the first report, much effort was expended in an attempt to eradicate the disease. By 1965, Tegethoff reported the eradication attempt to be successful. In 1982

Magasi and Pond reported the disease in southern New Brunswick. This was followed by a report of the disease in eastern Maine (Miller-Weeks and Stark, 1983). In 1985, Ostaff reported that subsequent surveys had found that the disease had been present in New Brunswick since at least 1958, and had since spread to Nova Scotia. It was present in 30% of the eastern larch stands surveyed and in some stands affected 100% of the trees. At this time it is impossible to state what effects the larch canker will have throughout the rest of the range of larch in North America. Yde-Andersen (1979) states in his literature review of the pathogen that there is no valid reason to assume resistance in the North American larch species. Goheen and Tkacz (1993) state that inoculation tests have shown that the disease is virulent to western larch. However, Ostaff and Newell (1986) found in limited trials that one to three year old western larch seedlings fared better than eastern larch or Eurasian larch species in disease resistance screening.

There have been reports of larch canker on Douglas-fir. The USDA, Forest Service (1991) states that it has been reported on Douglas-fir planted in Norway. There were several reports of the fungus on Douglas-fir prior to 1930. Hahn and Ayers (1934) made comprehensive studies of *Lachnellula* (at that time referred to as *Dasyscypha)* and found two species: one which attacked living larch and one, (*Dasyscypha calycina* Fckl.) which was a saprophyte on larch and Douglas-fir. Yde-Anderson (1979) states that in addition to *L. willkommii,* three species of *Lachnellula* occur on larch and sometimes other conifers. Both publications state that early reports of the larch canker on Douglas-fir are reports of related saprophytes. All of these tests were conducted with European isolates; little is known of the host range or

pathogenicity of East Asian isolates. The sunken, perennial cankers will likely be common and difficult to detect on imported larch logs (Goheen and Tkacz, 1993).

Scleroderris canker (*Gremmeniella abietina* [Lagerb.] Morelet) is another introduced canker disease. Although primarily a disease of pine, it has potential to affect larch. Two forms of the disease are present in North America; native strains affect seedlings and saplings under two meters tall, while European strains have the potential to kill trees of any size. Intermediate isolates have also been described. The European strain has been introduced into limited areas of the northeastern United States and eastern Canada, but it has only caused extensive damage in Upstate New York and eastern Newfoundland. The spread of this disease is currently being constrained by quarantine measures. Nursery tests with 42 species of conifer seedlings found eastern and Japanese larch to be slightly susceptible. Western, hybrid, European, Dahurian and Siberian larch (*Larix siberica* Ledeb.) were highly susceptible (Skilling et al., 1986).

Larch foliage is susceptible to several diseases. A recent, probable introduction is *Mycosphaerella laricina* (Hart.) Neg. This needle cast fungus of European larch was probably introduced on infested nursery stock to the Wilson forest nursery in Boscobel, Wisconsin and spread from there to nearby plantations as they were established (Patton and Spear, 1983).

Hypodermella larcis Tub. is also an important foliage fungus that causes a needlecast. It has a circumpolar, presumably native range. It has caused several epidemics of western larch and causes local damage on eastern larch in North America. It causes a disease of European larch in

Europe, but interestingly, not in North America. Foliage diseases caused by *Meria larcis* Vuill. and the *Melampsora* needle rusts are also ubiquitous and do not usually cause severe damage on larch (Hepting, 1971).

Larches and other genera of the Pinaceae share many of the common diseases caused by root and stem rotting fungi. Root rots caused by *Armillaria* spp. and *Heterobasidion annosum* are common on weakened trees. Their effects in North America are widespread, but not usually serious in vigorous stands of native larch (Hepting, 1971). In some instances, these root diseases can cause damage to plantations of non-native larch grown in the eastern United States (Robbins, 1985). In Eurasia however, annosus root disease is widespread and often serious in many larch stands (Hiley, 1919; Rozhkov, 1970; Hepting, 1971). Isolates from this area may have more pathogenic effects than native ones. Seedling inoculation tests have shown Douglas-fir to be very susceptible to a strain of *H. annosum* from pine in Scandinavia (USDA, Forest Service, 1991). This fungal species is also quite likely to be present and difficult to detect on imported conifer logs (Goheen and Tkacz, 1993).

Other root and heart rotting polypores with large host ranges such as *Phellinus pini* will probably also be present and difficult to detect in many imported logs. Once again, most of these fungal species are already present in North America. The primary cause for concern is the importation of virulent biotypes from an area where little is known of the fungal flora (USDA, Forest Service, 1991).

Vascular staining diseases of larch are considered of minor importance in North America. Hepting (1971) mentions only *Ophiostoma minus* (Hedg.) H. & P. Sydow = *Ceratocystis minor* (Hedg.) causing mortality in association with

infestation by bark beetles. Neither *O. polonicum* Siem. vectored by *Ips typographus* L. nor *Ceratocystis laricicola* Redfern & Minter vectored by *Ips cembrae* (Heer) are now present in North America. *I. typographus* is considered by Marchant and Borden (1976) to be the world's most dangerous bark beetle as an exotic pest invading new environments due to its extreme pest status in its native range, frequent interception in New Zealand and the U.K., numerous potential hosts and aggressive tree killing nature. Its associated stain fungus is one of the most pathogenic known, direct inoculation with a dose known to be lethal to Norway spruce (*Picea abies* [L.] Karst.) has also killed Douglas-fir and several North American spruces (Christiansen and Solheim, 1990). Although this pest complex is primarily associated with spruce, given the large volumes of larch considered for importation it is very likely to be present on imported unpeeled larch logs (USDA, Forest Service, 1991). The *I. cembrae-C. laricicola* complex was probably introduced into Britain in the late 1940's on conifer logs and has caused damage to larch plantations since then (Redfern et al., 1987). The beetle is common on larch timber throughout Russia (USDA, Forest Service, 1991). It is unknown if its associated stain fungus is also present, since it was only recently described.

Once again it is important to note that many of the pests that would be associated with larch logs can also affect other genera of the family Pinaceae. The larch genus is very similar in many respects to the Douglas-fir genus. Silen (1978) considers *Larix* to have contributed the most of any genera to the origin of Douglas-fir; possibly it is its direct ancestor. He based this conclusion on their striking similarity in: pollination mechanism, pollen, seed, cones,

seedlings, wood anatomy, bark, and shade intolerance. Presently, about half the standing timber in the western United States is Douglas-fir. It produces more timber than any other species in the nation and furnishes about 20% of the annual cut (Harlow et al., 1978). Although, many damaging agents affect Douglas-fir, virtually all are native and few are serious problems throughout its range or lifespan (Hermann and Lavender, 1990). Much of the public and professional scrutiny over larch log importation has been due to concern for the health of this species.

C. Mitigation Measures for Imported Logs

The purpose of this study was to identify measures for reducing the risk of microbial pest introduction from imported logs. The point of view that any risk is too great given the possible economic and environmental consequences cannot be ignored. However, this is a larger issue which must be decided by society as a whole. In order for society, through its policy makers to make an informed decision, the best scientific evidence should be made available.

A 1991 report by USDA, Animal and Plant Health Inspection Service (APHIS) reviewed the known effectiveness of various pest mitigation measures available to use on imported Russian logs. This report found many gaps in the scientific literature on the subject. For treatments to be approved by USDA, APHIS, data are required which demonstrate elimination of the pest hazard in the commodity. Treatments must also be supervised or verified by USDA, APHIS, and be in compliance with Federal and State environmental laws.

Mitigation measures reviewed but rejected for lack of basic efficacy data included: saltwater soaking, gamma irradiation, electron beam irradiation and microwave energy. Mitigation measures involving fumigants were more promising, but research was needed on wood penetration and toxicity against certain pests of concern. Heating was determined to be the only treatment known to be effective on all types of pests on and inside logs. Other mitigation measures were concluded to be useful as one part of a systems approach; they included: visual inspections, designated "pest-free" shipping areas, designated receiving ports, debarking and topical chemicals. Since some of these general measures may be used in combination with the lethal measures of heat or fumigants, they will be described prior to them. The measures requiring basic research will not be described.

1. Visual Inspections

Visual inspection is an important element of entry procedures because obviously infested material can be excluded entry or detained for additional treatment. The presence of bark greatly hinders the visual inspection process (USDA, APHIS, 1991). New Zealand Quarantine Officers sample an arbitrary 10% of each consignment of wood entering the country. High risk cargoes are sampled more intensively (Forest Research Institute, 1990b).

2. Shipping from "Pest-free Areas"

It would be reasonable to ship Siberian logs from areas known to be free from key pests of concern, such as active Asian gypsy moth epidemics. These areas would be subject to change as forest pest infestations change over time, space and intensity. Keeping this information current would require constant monitoring (USDA, APHIS, 1991). This measure was used in the past on North American oak logs exported to Europe. Oak wood from areas outside of the range of the fungus causing oak wilt (*Ceratocystis fagacearum* [Bretz] Hunt) did not require heat treatment. The European Community no longer considers this measure adequate (Liese et al., 1981). Although this measure will not be adequate in itself for Russian logs, it may be used as one part of a pest exclusion system (USDA, APHIS, 1991).

3. Designated Receiving Ports

Given the large number of suitable hosts in any receiving port city, and the wide host ranges of some of the pests of concern, such as the Asian gypsy moth, no port can be considered "safe". However, restricting the number of entry ports would facilitate the inspection and monitoring processes and may be one part of a pest exclusion system (USDA, APHIS, 1991). Under the Australian Quarantine Act logs may be imported into Queensland only through Brisbane, and all logs must be processed within 30 km of that port (Wylie and Yule, 1977).

4. Debarking

Most timber importing countries consider bark removal to be a sound practicable requirement that should be mandatory for logs or lumber. If retention of bark is considered necessary for special products such as veneer logs, fumigation or heat treatment is often compulsory (Tamblyn, 1963).

Thorough debarking can remove the majority of cambium feeding insects and superficial fungal infestations, but in actual practice bark often remains around large knots, pitch pockets, tree cankers and other irregularities. As many as 300 live *I. typographus* beetles have been intercepted on the remaining patches of bark on a single peeled Norway spruce log imported to Britain from Germany (Laidlaw, 1947). Of course, organisms deeper in the log are unaffected by bark removal. Frozen weather conditions at the time of debarking or poor mechanical condition of debarking devices can hinder the efficiency of this process. Timber imported to Norway from eastern Canada often has a "deplorably low standard of debarking" (Venn, 1986). USDA, APHIS (1993) requires that no more than 2% of the surface of all logs in a shipment may retain bark, and that no one log may have more than 5% of its surface retaining bark. Debarking would facilitate the inspection process and probably increase the effectiveness of fumigation (USDA, APHIS, 1991) or heat treatments.

5. Topical Chemicals

Pesticides such as pentachlorophenol or lindane, applied to the surface of a log can act as temporary protectants,

however penetration is insufficient to control pests established deep in the wood. Insecticides are commonly applied after felling to exclude pinhole borers from tropical logs bound for Australia (Tamblyn, 1963). USDA, APHIS (1991) recommends immersing Russian logs in pesticide after debarking at the shipping port to reduce the chances of pest escape before final lethal heat treatment at the receiving port, or lethal heat treatment at the shipping port followed by pesticide dip to prevent reinfestation prior to shipping. The agency also requires both a fungicide and an insecticide application on New Zealand logs bound for U.S. ports after debarking and fumigation (USDA, APHIS, 1993).

6. Heat

Heating via soaking in hot water, steaming, or kiln drying is known to be an effective means of eliminating pests both on and inside logs. It is considered the most promising method of those reviewed by USDA, APHIS (1991) for the treatment of Russian logs. Heat can be applied to logs as steam or hot water treatments, or kiln drying. The efficacy of heat treatments are dependant on temperature, wood moisture content, and wood volume (Siau, 1984).

Previous studies of heat treatments to control pests in wood have been summarized with respect to target organism in Tables 1, 2 and 3. It is readily apparent that steam and hot water treatments are more effective in killing organisms than dry heat. It is also apparent that certain fungi and the pinewood nematode (*Bursaphelenchus xylophilus* [Steiner & Buhrer] Nickle are more resistant to heat than the insects studied.

USDA, APHIS (1991) assumed that the schedule given by Jones (1973) to kill the oak wilt fungus would eliminate other fungal pathogens. It can be seen from the work of Newbill and Morrell (1991) and the recommendations of Rasmussen (1961) that more heat will be required to kill decay fungi. Furthermore, they assume that steam will be equally effective as immersion in hot water. Kinn (1986) has shown this not to be the case in his studies with the pinewood nematode.

Heating would require extensive facilities and large energy expenditures at the shipping point. These facilities would require large capital investments in the unstable political and economic climate of contemporary Russia. These considerations make this treatment technique financially risky.

Table 1. Heat required to kill fungi in wood.

Target species	Temp. °C	Time	Conditions	Source
stain and decay fungi	66	24 hours	green wood in dry kiln	Rasmussen (1961)
Ceratocystis fagacearum	43	48 hours	green logs in heat chamber	Jones (1973)
"	54	24 hours	"	"
"	43	24 hours	green logs immersed in water	"
"	49	12 hours	"	"
Peniophora spp.	49	30 minutes	wood blocks in plastic bag immersed in water	Newbill and Morrell (1991)
Stereum sanguinolentum	49	75 minutes	"	"
Postia placenta	66	75 minutes	"	"
Antrodia carbonica	66	75 minutes	"	"

Table 2. Heat required to kill the pine wood nematode in wood.

Temp. °C	Time	Conditions	Source
60	5 hours	green wood chips dry heated	Dwinell (1986)
50	13 hours	"	"
135	10 minutes	"	Kinn (1986)
85	2 minutes	green wood chips steam heated	"
65	8 minutes	"	"
55	2 minutes	green wood chips immersed in water	"
50	6 minutes	"	"
71	4 hours	15 cm squared lumber in kiln	Dwinell (1990)

Table 3. Heat required to kill wood boring insects.

Target species	Temp. °C	Time	Conditions	Source
Xylotrechus quadrupes	60	5 minutes	unknown	Uvarov (1931)
"	50	60 minutes	"	"
Anobium striatum	46	1 minute	"	"
Lyctus brunneus	45	1 minute	"	"
Monochamus spp.	71	1 hour	5 cm thick softwood lumber	Ostaff and Cech (1978)

7. Fumigation

Fumigation would require fewer facilities and could be accomplished on ships or in port. Fumigants are chemicals which are volatile at ordinary temperatures and toxic enough to provide an acceptable level of control against pest species (Monro, 1969). Morrell (1990) makes the distinction between long-term wood fumigants and short-term wood fumigants. Long-term fumigants such as Vapam, Vorlex and chloropicrin move slowly through wood and remain for three to 17 years, providing lasting protection. Although Vapam has been tested with success in eliminating the pine wood nematode from Southern pine wood chips in a laboratory study (Kinn and Springer, 1985), it and other long-term fumigants are not likely to be used for treatment of raw imported logs or other large pieces of wood. Timber treated by this method would be unsuitable for uses without adequate ventilation or

where direct human contact was likely to occur due to the long-term volatilization of toxic chemicals (Morrell, 1990).

Short-term fumigants such as phosphine, sulfuryl fluoride, methyl bromide and to some extent hydrocyanic acid are much more volatile compounds that rapidly penetrate the wood, eliminate pests and escape into the atmosphere. The principle fumigants being proposed for the treatment of raw logs are short term fumigants which rapidly dissipate after treatment.

a. Theoretical Wood Permeability

Fluid flow is analogous to heat transfer in wood. The mechanisms of gaseous and liquid flow differ from that of heat, but are in concept the same:

$$\text{CONDUCTIVITY} = \frac{\text{FLUX}}{\text{GRADIENT}}$$

Conductivity of fluids through a porous substance is termed permeability. Permeability is a function of wood anatomy, and can vary within and between species, as well as within an individual trees.

Permeability is important in wood technology because it governs the movement of water out of wood, which in turn affects drying. Also, it governs the reverse, movement of water and other compounds such as fumigants into the wood. This has important implications for application of chemicals in wood preservation, fumigation and other treatments.

The 1991 USDA, APHIS review of control measures for potential pests of imported Soviet timber stated: "Siberian

timber species being proposed for import are all conifers (softwoods) and should pose even less of an obstacle to penetration by the gas than does oak (a hardwood)". This is not the case, although it illustrates the importance of making the distinction between permeability and porosity. Porosity is defined as the volume fraction of void space in a solid (Siau, 1971). Since cell wall components are very similar for all wood species (cellulose, lignin and hemicellulose), porosity is largely density dependant. To be permeable a substance must be porous, but not all porous substances are permeable. For instance, northern red oak (*Quercus rubra* L.) is denser, and therefore less porous than eastern larch. However, red oak is much more permeable than eastern larch (Hunt and Garrett, 1967). This difference reflects the large unobstructed vessel elements of red oak in contrast to the smaller obstructed tracheids of eastern larch.

The permeability of wood is a function of the variables of wood anatomy and fluid flow. There may be as many as three different kinds of flow in wood, laminar or viscous flow, turbulent flow, and slip or Knudsen flow. Due to the small diameters of the softwood tracheids, the effect of capillarity should also be accounted for. In addition, flow can be either steady-state or unsteady-state. Steady-state flow exists when the flux and gradient do not change over space and time; unsteady-state flow is variable in these respects. Unsteady state flow is more relevant for wood treatments, because processes such as drying, and preservative or fumigant application require a change in conditions within the wood over time. The following discussion of fluid flow in wood is adapted from Siau (1971).

Laminar flow is the most important type of flow through wood. During laminar flow the viscosity or internal friction of the fluid requires the exertion of a force that causes one layer to flow smoothly past another. Laminar flow occurs with both liquids and gases. Steady state laminar flow of liquids is governed by Darcy's law:

$$k = \frac{V\ L}{t\ A\ \Delta P}$$

where:
k = permeability, cm^3 (liquid)/cm atm sec
V = volume of liquid flowing through specimen, cm^3
t = time flow, sec
L = length of specimen in direction of flow
A = cross sectional area of specimen perpendicular to flow, cm^2
ΔP= pressure difference between upstream and downstream ends of specimen, atm

Steady state laminar flow of gases must take into account the changes in gradient due to the expansion of gases. This requires that Darcy's law be written in derivative form:

$$k_g = - \frac{V\ L\ P}{t\ A\ \Delta P\ (P_2+P_1)/2}$$

where:
k_g= superficial gas permeability, cm^3/cm atm sec
V = volume of gas flowing through specimen, cm^3
P_1= pressure of upstream end of specimen, atm
P_2= pressure of downstream end of specimen, atm

Comstock (1967) found that the permeability of wood is a characteristic of wood anatomy and is independent of the fluid as long as the fluid does not swell the wood. In contrast, rate of flow is dependant on the viscosity of the

fluid. Rates of flow can be calculated by using Poiseuille's law of viscous flow:

$$Q = \frac{\pi R^4 \Delta P}{8 \eta L}$$

where:
Q = rate of flow, cm^3/sec
R = radius of capillary, cm
η = viscosity of fluid, $(dyne\ sec)/cm^2$

Turbulent flow occurs as laminar flow breaks down at high velocities. It is not known if this type of flow occurs in wood. It may possibly occur in the passages of the pit openings, where the flow is constricted and velocities increase, but is usually disregarded in flow calculations of wood.

Knudsen or slip flow occurs only in gases along the surface of a capillary, where according to Darcy's law the flow should be zero. Slip flow is only important when the radius of the capillary is very small relative to the mean free path of the gas molecules. This is the average distance of travel between the colliding gas particles, and it varies directly with temperature and pressure. The mean free path is calculated by the following equation:

$$\lambda = \frac{2\eta}{(P_2+P_1)/2} \sqrt{\frac{\Re\ T}{M_w}}$$

where:
λ = mean free path, cm
$\Re$ = universal gas constant = 8.31×10^7 erg/(mole°K)
M_w = molecular weight of gas

This result can be combined with Darcy's law in the Klinkenberg formula:

$$k_g = k\left(\frac{1 + 3.8\,\lambda}{R}\right)$$

where:
k_g= gas permeability, including laminar and slip permeability
k = longitudinal or transverse permeability without slip flow
R = radius of pit openings, cms

Capillarity is another factor to be considered when a liquid-gas interface is present. Surface tension can be viewed as a membrane; force must be applied to move the meniscus through a capillary. Surface tension can be calculated by the following formula:

$$\gamma = \frac{R\,\rho\,g\,h}{2\cos\theta}$$

where:
γ = surface tension or surface specific energy, dyne/cm
R = radius of capillary, cm
ρ = density difference between liquid and gas, gm/cm^3
g = gravitational acceleration = 980 cm/sec^2
h = height of capillary rise, cm
θ = contact angle of meniscus and capillary

According to Stamm (1966) this equation holds for irregular as well as circular pores and even triangular openings and slits.

Unsteady-state flow is of great importance in wood treatments, because in actual practice there is a change in conditions over time. It is very difficult to derive a formula that will account for all of the variables involved in the complex interactions of permeability. Some of these are: viscous, slip and capillary forces; polarity of the fluid; treatment time; specimen length and shape; and back

pressure of air in the wood. Unsteady-state water flow in a wood slab can be estimated by Fick's second law:

$$\frac{\delta M}{\delta t} = \frac{100\ K_d}{G\ \rho_w} \frac{\delta^2 M}{\delta x^2}$$

where:
M = moisture content, percent
ρ_w= density of water, (1 gm/cm^3)
G = specific gravity of moist wood
K_d= conductivity coefficient for moisture movement, gm/(cm % sec)
x = distance in flow direction, cm

For gaseous flow in a slab the unsteady-state flow is derived from Darcy's law for gases:

$$\frac{\delta m}{\delta t} = - \frac{k_g\ A\ M_w}{2\ \Re\ T} \left(\frac{\delta P^2}{\delta x} \right)$$

where:
m = mass of gas transferred either in or out of the slab, gm
k_g= superficial gas permeability
P^2= upstream pressure at distance x, atm

It should be apparent that existing mathematical formulas to predict fluid flow in softwoods are simplifications that would be most accurate under tightly controlled laboratory regimes. The formulas to estimate permeability consider capillary (tracheid, resin duct, pit) radius or cross sectional area, and length or height. It would be very important to have estimates based on accurate measurements of these capillary dimensions in order to obtain accurate flow predictions.

b. Effects of Softwood Anatomy on Permeability

Tracheids are the primary constituents of softwood xylem. Panshin and de Zeeuw (1970) estimate that eastern white pine (*Pinus strobus* L.), a typical softwood is 93% tracheids, 1% resin canals and 6% rays. Tracheids are also the principle component of rays. Consequently, tracheids are also the primary means of fluid permeability in softwoods. Their predominantly longitudinal orientation results in permeability in that direction being from 10,000 to 40,000 times as great as transverse permeability (Comstock, 1970). The effective permeability in both directions varies largely with tracheid variables such as: lumen radius, length, number of pits, effective pit diameter, pit orientation, pit aspiration, and occlusion with extractives or lignin-like substances.

The term lumen radius is an assumption in many cases because the shape is closer to that of a rounded square or an ellipse. The error introduced in calculations by this assumption is not thought to be excessive (Stamm, 1946). Siau (1971) states this dimension to be about 10-15 microns, and tracheid length in softwoods varies from about 1.2 to 7.5 mm. Stamm (1970) found maximum tracheid length in eastern larch samples to be 5.0 mm and maximum lumen radius to be 16 microns. Latewood lumen radii are generally smaller than those of earlywood.

The lumens of larch tracheids and ray cells often contain extractives. The most common extractive in larch wood is the water-extractable polysaccharide gum arabinogalactan which occurs in the lumens as an amorphous mass of extracellular material. The heartwood of all larch species contain 5-35% arabinogalactans on a dry weight basis.

The butt logs of western larch trees often contain so much arabinogalactan that they are good for neither pulping nor sawtimber. The concentration in all commercially important larch species is lowest in the pith and increases radially to a maximum at the sapwood heartwood boundary (BeMiller, 1989b). A positive relation between the percentage of heartwood and the content of arabinogalactan was found in Dahurian larch (BeMiller, 1989a). Imamura (1989) reported the extractive contents from the wood of 17 species of conifers. Of these, Dahurian larch and Japanese larch had the highest concentrations of water soluble extractives (11.4% and 3.9-20.1%, respectively), and the sixth and second highest concentrations of ethanol soluble extractives (3.0% and 1.8-5.5%, respectively). These high extractive contents tend to result in wood that is dense (0.58 and 0.53, respectively) and has low porosity.

Intercellular flow is largely through the pits. In most softwoods there are usually about 50-300 bordered pits per tracheid; most of these are arranged on the radial face and near the ends of the tracheid (Stamm, 1946). Pit chambers in Dahurian larch have a radius of about 4-5 microns (Greguss, 1955). Latewood pits are usually smaller than earlywood pits. The torus is one half to one third of the diameter of the pit chamber, and the diameter of the aperture is about half the diameter of the torus. The margo is composed of microfibril strands, and passage of fluids and minute solids occurs through these openings. The effective radii of these pit openings are between 0.01 and 4 microns. Latewood has been found to have thicker tori than earlywood (Siau, 1971).

Bordered pits can often become aspirated, particularly in heartwood. This occurs when the torus becomes closely appressed to one side, effectively sealing the passage.

Aspiration is more common in earlywood than in latewood due to the thicker less flexible torus in latewood. Earlywood pits often aspirate, particularly during the drying process, causing uneven permeability. In the heartwood of species with high extractive content such as Dahurian larch, pits can often become occluded with extractives. Also, pits can become encrusted with insoluble lignin-like substances (Siau, 1971). Aspiration of heartwood, along with occlusion and encrustation are the reason for its impermeability relative to sapwood. Erickson et al. (1937) found inland Douglas-fir (*Pseudotsuga menzesii* Mirb. Franco var. *glauca*) sapwood to be 518 times as permeable as the heartwood. Stamm (1970) found the effective pit pore radius of green eastern larch sapwood to be 33.3 times greater than that of heartwood. Since rate of flow varies to the fourth power of effective pit pore radius, he calculated fluid flow in sapwood of eastern larch to be approximately 124,000 times greater than heartwood.

Although relatively uncommon, simple pits contribute to permeability. Since they have no torus, they cannot aspirate. They occur primarily in parenchyma cells; parenchyma is uncommon in Dahurian larch (Greguss, 1955). The openings for the plasmodesmata are between 0.05 and 0.3 microns, and could act as simple pit pairs, (Siau, 1971). Stamm (1946, 1963) stated that fluids could flow through water saturated cell walls of tracheids between the cellulose microfibrils. He stated this to be an important source of flow in swollen wood, but Siau (1971) did not consider this passage to be important.

The resin ducts or canals of certain softwoods also contribute to permeability, but the magnitude of this contribution is variable. Stamm (1946) considers them to be ineffective to fluid transmission due to obstruction with

resin. However, Erickson (1938) found resin canals to be the most effective component of softwood anatomy with respect to radial permeability, but there was a very small rate of flow when the resin canals were sealed with resin or tylosoids. The wood samples he investigated were 1.25 mm thick tangential sections. He stated that the percentage of unobstructed resin canals through thicker sections was likely to be small. Greguss (1955) measured the diameter of resin canals of Dahurian larch wood samples and found them to be 50-60 microns.

From the preceding discussion it should be obvious that permeability of wood to fumigants is difficult to accurately predict. The complex anisotropic nature of a particular wood species or specimen, and environmental variables over time and space make most predictors either unreliable or very specific. In treatment practice wood species are usually placed into general categories of permeability with respect to the treatment process. Dahurian larch heartwood has been tested and found to be difficult to permeate with waterborne preservatives (Morrell and Schneider, 1994). Permeability studies with fumigants are lacking, although estimates can be made by comparing fumigant permeability of other wood species.

c. Effects of Moisture Content and Temperature

In addition to wood size and anatomy variables, fumigant penetration in wood is very dependant on the moisture present in these capillaries which can impede penetration by acting as a physical barrier or generally increasing sorption of the gas, particularly gases with any degree of solubility.

Fumigants with higher boiling points are more likely to be sorbed than more volatile ones (Munro, 1969). Since the logs proposed for importing will be green, it is important to obtain a reliable estimate of their moisture content for heat or fumigation treatment. Isaeva (1966) studied the distribution of moisture content of six living Siberian larch trees by extracting 27 cores from each. Moisture content was distributed as follows:

Table 4. Moisture content distribution in Siberian larch trees.

	sapwood	heartwood	pith
mid crown	130%	45-60%	50%
mid trunk	115%	45-60%	60%
stump	95%	40-50%	45%

It is important to remember that rotten and diseased areas on logs commonly have much higher moisture contents than unaffected areas.

The temperature of the wood is also an overriding consideration which directly dictates the vapor pressure or expansiveness of the fumigant. Also, sorption by wood is greater at lower temperatures. If fumigation is impractical with cold or frozen logs, then heat must be provided at some point or exports may only be permitted during periods when the log temperatures are above freezing (USDA, APHIS, 1991). Consideration of these variables is essential when estimating penetration of fumigants.

d. Short Term Wood Fumigants

(1) Hydrocyanic Acid

Hydrocyanic acid (HCN, Zyklon B) was the first fumigant used to destroy pests in wood. It has a molecular weight of 27.03, a boiling point of 26.0° C at normal atmospheric pressure, is very soluble in water at 20° C and is flammable in air in concentrations of 6-41% by volume (Munro, 1969). Whitney (1961) reports its specific gravity to be 0.688 as liquid and 0.93 as gas, and to have a vapor pressure of 739 mm at 25° C.

Kemner (1915, from Harris 1963) reported successful fumigation against *Anobium* larvae in wood up to 4-5 cm deep using a dosage of 10 mg/l for 12-15 hours. Temperature, wood species and wood moisture content were not mentioned. Parkin and Busvine (1937) reviewed the results of Nagel (1921) who investigated the resistance of *Anobium* larvae *in vitro* to HCN. Mortality was complete after exposure to: 11.2 mg/l for 2 hours, 5.6 mg/l for 4 hours or 1.1 mg/l for 24 hours. However, these results were considered excessive by the reviewers due to leaks in the testing apparatus. For fumigation in wood using a partial vacuum of 40-50 cm of Hg to ensure penetration, 34-45 mg/l of HCN killed all larvae within 24 hours. Once again leakage confounded the results when the vacuum fell to zero after 10 hours. Larvae were unaffected by vacuum alone.

Parkin and Busvine (1937) also mention the successful fumigation of a church attacked by *Anobium*, reported by Oberwalder et al (1930), and an anonymous Danish contribution using HCN against the Cerambycid *Hylotrupes bajulus* L. In their own detailed, controlled work, they determined the

toxicity of HCN to *Lyctus* larvae at 20° C and 25° C, and both *Lyctus* and *Anobium* larvae at 25° C. Harris (1963) reviewed the work of Parkin and Harris (1939) who determined the efficacy of commercial scale fumigations with HCN against wood borers, and that of Bletchy (1953) who determined the concentration X time product (CxT) necessary to give a 100% kill of *Anobium* eggs at 25° C.

More recent work by Unger (1983, abstract only) demonstrated the feasibility of using HCN to destroy wood borers and brown rot fungi in the Scots pine (*Pinus sylvestris* L.) timber framework of a historical building. Treatment efficacy was monitored by fitting wood samples (some end coated) containing wood borers (*H. bajulus, Anobium punctatum* [De Geer] and *Dendrobium pertinax* L.) and the wood rotting fungus *Serpula lacrimans* (Wulf. ex Fr.) Lind. to beams in the building as a bioassay and vibration sensors to detect insect activity. Fumigation consisted of 30 mg/l of HCN for 72 hours. At the end of fumigation all test fungi and almost all test larvae were killed by the treatment; no movement of larvae could be detected. Only *Anobium* larvae in end coated wood samples survived.

Kramer (1992) summarized the advantages of HCN as being a fast, easy to apply, yet highly toxic fumigant. The chief disadvantages were its explosive nature and very poor penetration in materials with high moisture contents due to its solubility. Harris (1963) also noted poor penetration in large timber, particularly where end penetration was prevented, often necessitating the use of a vacuum chamber. For this reason, it has greater logistical drawbacks than heating that make HCN or vacuum fumigation for that matter, unsuitable for treating large wet logs.

(2) Phosphine

Phosphine or hydrogen phosphide (PH_3) has also been used to destroy pests in wood. It is normally used in the form of aluminum or magnesium phosphide tablets that give off phosphine gas in the presence of moisture. It has a molecular weight of 34.04, a boiling point of -87.4° C, is very slightly soluble in water, and highly inflammable (Munro, 1969) when in concentrations above 1.79% in air (USDA, APHIS, 1991). Its specific gravity is 1.2 as a gas, and Whitney (1961) lists the vapor pressure at normal atmospheric pressure as being "very high". Since its specific gravity is near that of air, fans or blowers to aid mixing are unnecessary. Colorimetric detector tubes are used to monitor concentration levels, because flame detectors could possibly ignite the gas (USDA, APHIS, 1991).

Richardson (1974) found 0.7 mg/l of phosphine for 72 hours gave complete mortality against a subterranean termite *Nasutitermes exitiosus* (Hill) nest in a one liter glass jar, and three species of flour beetles in an 8x8x20 mm cavity of a 150x100x50 mm radiata pine block. Seventy-two hour fumigations of 1.06, 1.41 and 2.1 mg/l against the *Sirex noctilio* F. wood wasp in small pine logs resulted in 6, 94 and 94% mortality, respectively. Temperature never dropped below 8° C in any of the tests.

Unger et al. (1984, abstract only) used phosphine to exterminate the wood boring beetles *A. punctatum* and *D. pertinax* from an 18th-century church at 0.23 to 0.30 mg/l. Although time of fumigation was not given, the authors noted that the fumigation should be well sealed and performed above 15° C. Li et al.(1988, abstract only) found phosphine

fumigation to be effective in controlling a pine sawyer, *Monochamus sutor* L. in Dahurian larch logs.

Leesch et al. (1989) tested phosphine in an in transit shipboard fumigation to control the pine wood nematode in Southern pine woodchips. 4 mg/l phosphine was applied at the beginning of the voyage; after seven days all the gas had escaped. Temperature of the woodchips varied from 20° C to 47° C. Bioassays revealed that 4 of 68 samples had live nematodes compared to 38 of 48 prior to fumigation. Nematode populations commonly increase several-fold during transit (Dwinell, 1987). This occurs around the edges of chip piles; the heat generated in the center of piles probably kills the nematodes there (Dwinell, 1986).

Although phosphine is comparatively safe and easy to use, its drawbacks are the long fumigation times necessary, its tendency to react with copper in electrical systems, the potential fire hazard of using high concentrations and the resistance of some insect species (Kramer, 1992). Also, studies to determine its fungitoxicity and ability to penetrate large wet logs are necessary before it can be completely evaluated (USDA, APHIS ,1991).

(3) Sulfuryl Fluoride

Sulfuryl fluoride (SO_2F_2) is a colorless, odorless fumigant used mainly in structural fumigations of termite infested buildings. It has a molecular weight of 102.6, a boiling point of -55.2° C, is slightly soluble in water and nonflammable (Munro, 1969). The specific gravity as a gas is 3.52 and the vapor pressure is 13,442 mm at 25° C. This extremely high vapor pressure aids gaseous penetration of

commodities and promotes rapid attainment of gas-air equilibrium (Kenaga, 1957). Air circulation with fans or blowers is only necessary for the first hour of fumigation. Gas concentrations are monitored during fumigation with a thermal conductivity detector (USDA, APHIS, 1991).

Penetration in dry wood is usually excellent. Stewart (1957) using the drywood termite (*Incisitermes minor* [Hagen]) as a bioassay found that conifer sawdust was penetrated much more rapidly by sulfuryl fluoride than methyl bromide. He concluded that methyl bromide was sorbed more by the sawdust. Kenaga (1957) also found sulfuryl fluoride to be more effective than methyl bromide in killing black carpet beetles (*Attagenus piceus* [Oliv.]) and confused flour beetles (*Tribolium confusum* Duv.) buried 13-23 cm in hardwood flour. Bess and Ota (1960) found sulfuryl fluoride to be "far superior" to methyl bromide when testing both fumigants against the drywood termite *(Cryptotermes brevis* Walker) in 9x9x15 cm fir wood blocks placed in fumigated buildings. Although both fumigants adequately controlled natural termite infestations and termites in petri plates in fumigated buildings, sulfuryl fluoride killed all termites in all test blocks in all 8 buildings, while methyl bromide only killed all termites in all test blocks in 6 of 18 building fumigations.

Similarly, Su and Scheffrahn (1986) found mortality of three termite species was reduced if insects were sealed in slash pine (*Pinus ellotii* Engelm.) heartwood block cages as opposed to petri plates during sulfuryl fluoride fumigation. Mortality was particularly reduced for the Formosan subterranean termite (*Coptotermes formosansus* Shiraki) due to premoistening of the enclosure to ensure this species did not desiccate. Scheffrahn et al. (1992) found sulfuryl fluoride

penetrated dry wood samples of all five species tested better than methyl bromide in 20 hour fumigations of 16 mg/l (CxT = 320). Penetration of both fumigants was about equal for Southern pine wood samples hydrated to 30-35% moisture content which significantly reduced penetration of both gases. Longitudinal diffusion in dry (9.3% moisture content) Southern pine was extremely rapid for both fumigants. Douglas-fir and red oak had the least transverse permeability of the species tested for methyl bromide and sulfuryl fluoride penetration, respectively. In contrast, Scheffrahn and Thomas (1993) reported methyl bromide penetrated vinyl coated nylon and polyethylene tarp materials to a greater extent than sulfuryl fluoride; this indicates that methyl bromide fumigations may require closer monitoring than sulfuryl fluoride.

Fungitoxicity data for sulfuryl fluoride has been summarized in Tables 5 and 6. Su et al. (1989) tested various concentration and time combinations of sulfuryl fluoride on the Formosan subterranean termite and found exposure time and concentration of fumigant to be equally important and virtually interchangeable. Su and Scheffrahn (1986) consider the CxT values reported by La Fage et al. (1983) to be excessive because mortality counts were taken at 24 hours instead of 72 hours. However, Stewart (1957) in his studies with drywood termites reported that although both fumigants appear equally toxic, delayed mortality was more common with methyl bromide.

Stewart (1957) also reported that sulfuryl fluoride was more sensitive to temperature change, being slightly more toxic than methyl bromide above 13° C and less toxic below that temperature. His 1962 study found 6.1 times as much sulfuryl fluoride was necessary to kill drywood termites at

4.5° C than at 21° C. LaFage et al. (1983) found similar results for the Formosan subterranean termite. Kenaga (1957) found increasing exposure times by approximately the same margin necessary for both fumigants to achieve the same mortality with a fixed concentration of gas as temperature was reduced.

He also reported the difference in susceptibility between egg and adult life stages of five insect species to sulfuryl fluoride. Eggs of the insects investigated required from 4 to 54 time as much exposure to sulfuryl fluoride for 95% mortality than did adults. USDA, APHIS (1991) does not endorse the use of sulfuryl fluoride on Russian logs due to the relative ineffectiveness of the fumigant against insect eggs and the lack of efficacy data with respect to specific plant pathogens and nematodes.

Table 5. Toxicity of sulfuryl fluoride to insects, primarily termites as determined by laboratory studies.

Target Species	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Kalotermes minor	21	64	96-100	in petri plates	Stewart 1957
"	21	106	94	under 28 cm of sawdust	"
"	4.5	394	100	in petri plates	Stewart 1962
Tribolium confusum	27	128	100	under 23 cm of hardwood flour	Kenaga 1957
Attagenus piceus	"	128	100	"	"
Coptotermes formosansus	30	132	100	in plastic cups	La Fage et al. 1983
"	10	238	12	"	"
Seven termite species	27	20-51	99	in petri plates	Osbrink et al. 1987

Table 6. Toxicity of sulfuryl fluoride to insects, primarily termites as determined by field studies.

Target Species	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Cryptotermes brevis	22-27	544	100	3.2 cm thick fir blocks	Bess and Ota 1960
Cryptotermes brevis	20-24	180	100	2.0 cm thick pine blocks	Minnick et al. 1972
Tropical insects of 6 families	?	1920	100	3 species of tropical hardwood logs	Tang et al. 1985
Cryptotermes cavifrons	27	69	100	3.8 cm thick pine blocks	Su and Scheffrahn 1986
"	"	44	"	in petri plates	"
Incisitermes schwarzi	"	46	"	3.8 cm thick pine blocks	"
"	"	43	"	in petri plates	"
Coptotermes formosansus	"	95	"	3.8 cm thick pine blocks	"
"	"	46	"	in petri plates	"

(4) Methyl Bromide

Methyl bromide (CH_3Br) is one of the most versatile and effective fumigants due to its ability to penetrate quickly and deeply into sorptive materials at normal atmospheric pressure (USDA, APHIS, 1991). This colorless and odorless gas has a molecular weight of 94.95, a boiling point of 3.6° C at normal atmospheric pressure, a solubility in water of 13 gm/l at 25° C and is nonflammable. It has specific gravities of 1.732 as a liquid at 0° C and 3.72 as a gas at 0° C. Its vapor pressure is 1390 mm at 20° C (Munro, 1969) and its viscosity at 0° C is 0.397 cp (Windholz et al., 1976). The use of methyl bromide and some other halogenated hydrocarbons will likely be phased out in the near future due to concerns with their possible role in ozone depletion of the atmosphere (Kramer, 1992).

Because it is much heavier than air, methyl bromide diffuses quickly laterally and downward, but upward slowly. Circulation is essential to ensure thorough fumigant distribution and penetration. A volatilizer is also required when applying the fumigant to keep gas lines from freezing. As with the other fumigants, commodities to be fumigated must be enclosed in a chamber or tarpaulin to retain fumigant for the desired time period (Munro, 1969).

During fumigation, methyl bromide concentrations may be monitored with halide thermal conductivity analyzers, sampling tubes or changes in the flame of a halide leak detector. The flame of the detector turns from yellow to green and then blue with increasing fumigant concentrations (Munro, 1969). The New Zealand Plant Protection and Quarantine Service uses gas permeable sachets containing an absorbent for methyl bromide in one compartment and a reagent

in another, placed in a shipment of timber to be treated. Upon mixing the two contents, a distinctive color is produced if the sachet has been exposed to a preselected minimum methyl bromide concentration time product (Cross, 1991).

The effectiveness of methyl bromide as a commodity sterilant is due to its toxicity and its penetrating ability. Although this penetrating ability is reduced as the gas is sorbed by the commodity (Munro, 1969), Michelsen (1964) showed methyl bromide diffusion and sorption rates by wood both vary inversely with moisture content. He investigated samples of pine wood below fiber saturation point (moisture contents between 4 and 17%). Wood at 17% moisture content held 5 times as much methyl bromide as the surrounding air in the fumigation chamber, while wood at 4% moisture content sorbed 37 times as much.

Michelsen also estimated theoretical diffusion coefficients for methyl bromide in pine wood at 14-17% moisture content and 24° C. He compared the graphically estimated amount of gas sorbed into rectangular wood samples (four sides effectively sealed) over time with the graphically derived maximum amount the wood samples would sorb. Longitudinal diffusion of sapwood was 14.0-23.4 cm^2/hr, while longitudinal diffusion of heartwood was 3.0-6.0 cm^2/hr. Radial diffusion was much less, 0.44-0.85 cm^2/hr for sapwood and 0.46-0.79 cm^2/hr for heartwood. Due to the large amount of extrapolation used in deriving these numbers, they should be regarded with caution.

In another diffusion study, Ricard (1966) detected methyl bromide in the piths of nine 20-48 cm diameter Douglas-fir utility pole sections from 71-152 cm long (ends sealed with paraffin) in half an hour at fumigant concentrations of 160 mg/l with the temperature from 12-26°C.

The applicability of this study is questionable since the dry, checked, debarked pole sections were cut from poles that had been in service. It is also likely that the paraffin coating on the ends of the pole sections did not entirely hinder longitudinal penetration. USDA, APHIS (1991) refers to these pole sections as logs. This is not the case; Ricard (1966) states in his dissertation that they were used pole sections that had been in service. However, Ricard et al.(1968) do not make that distinction clear in their more widely disseminated publication.

Liese et al. (1981) studied penetration by methyl bromide of effectively end coated red and white oak log sections 35-45 cm long and 24-39 cm in diameter with sapwood 2.1-8.3 cm thick and 0.4-1.3 cm bark on. The wood was from freshly fallen trees with sapwood moisture contents of 80-90%. They performed 20 fumigation experiments at concentrations of 80-270 mg/l for 72 hours and temperatures of -3, 5 and 9° C.

They found diffusion to vary both between and within species, red oak sapwood being penetrated more easily than white. The outer sapwood attained higher gas concentrations than the inner sapwood, however chemical levels rapidly diminished as the fumigation was terminated. Diffusion in the inner sapwood was delayed and concentrations did not reach levels as high. However, these concentrations were maintained longer; even 26 hours after a 110 mg/l fumigation was terminated, 20 mg/l was detected 4 cm deep in the sapwood. This effect was more pronounced at higher initial gas concentrations. Temperature was also an important variable. A fumigation conducted at 5° C was found to result in a 30% greater CxT product at 4 cm depth than one conducted at -3° C; the fumigations were otherwise identical. Initial

concentration was also an important factor in penetration of gas. A fumigation with an initial concentration of 185 mg/l at 5° C was found to give methyl bromide concentrations 4 cm deep in the sapwood about twice as great as one conducted at 9° C with an initial concentration of 110 mg/l. A large amount of the penetrated gas was sorbed by the wood, approximately 15 times the amount in the air at 5° C. The study did not demonstrate effective gas penetration deeper than 7 cm into the wood.

In contrast to the fairly rapid diffusion values reported above for dry pine wood, Cross (1991) found penetration of green radiata pine (*Pinus radiata* D.Don) sapwood to be a much more difficult task. His fumigation tests were performed on log sections with all cut surfaces effectively sealed to allow only radial diffusion through the bark. Fumigations of 12 and 24 hours were conducted at gas concentrations of 260 mg/l on green blocks and 6 hours at 160 mg/l on dry blocks. All fumigations were performed at 20-25° C.

His data showed that amounts of methyl bromide reaching the sampling points over time decreased rapidly with increasing penetration distance. Also, the amount that did diffuse through was delayed in proportion to the thickness of the wood. Methyl bromide did not arrive at the 10 cm sampling point until 48 hours had elapsed, reaching maximum concentrations in 80-90 hours at less than 20 mg/l and falling away to trace amounts at the end of a week. The gas in the outer layers of the wood served as a reservoir that was available for continued penetration with decreasing effect due to losses by diffusion out of the timber.

A curvilinear gradient for methyl bromide penetration into green timber and a linear one into dry timber was

reported, due to decreased sorption in dry wood compared to green wood. This appears to contradict the findings of Michelsen (1964), but he restricted his investigations to wood below fiber saturation point. The gradient for green timber was such that it was found to be impractical to achieve insecticidal CxT products much beyond a depth of 10 cm in radiata pine sapwood using conventional tent fumigation techniques.

Although methyl bromide was first used as an insecticide in 1932 by Le Goupil (Monro, 1969), controlled studies of its toxicity to insects pests in wood are relatively few. The studies conducted by Stewart (1957), Kenaga (1957) and Bess and Ota (1960) comparing insecticidal efficacy of methyl bromide to sulfuryl fluoride have been described above. Harris (1963) and Cross (1991) reported CxT values for common wood boring insects of Europe and New Zealand, respectively. Munro (1969) points out the great effect that temperature can have on the success of fumigation for insect control. Since insects respire more at high temperatures, they take in more fumigant. Sorption of the gas by the commodity is also less at higher temperatures, as was mentioned previously.

The CxT values of the field studies listed are several times those listed for laboratory studies. This is to ensure adequate penetration of large timbers necessary to achieve complete mortality. Monitoring these studies involved test insects placed in sample blocks. Mortality of the complete object was assumed from these samples and subsequent inspection of the object.

Although USDA, APHIS (1991) considers methyl bromide to be an effective nematicide, studies on its efficacy against *Bursaphelenchus* spp. are lacking. Further research is

necessary before the agency can grant approval for its use in treating logs infested with nematodes.

Table 7. Toxicity of methyl bromide to insect pests, primarily wood boring insects as determined by laboratory studies.

Target insect	Temp. °C	CxT mgh/1	Mortality %	Conditions	Author
Kalotremes minor	21	64	96-100	in petri plates	Stewart 1957
"	"	115	7	under 28 cm of sawdust	"
Attagenus piceus	27	128	82	under 2.5 cm of hardwood flour	Kenaga 1957
Tribolium confusum	"	"	100	"	"
Attagenus piceus	"	"	0	under 23 cm of hardwood flour	"
Tribolium confusum	"	"	6	"	"
Anobium punctatum	15	190	100	in plywood	Harris 1963
Xestobium rufovillosum	"	200	100	in oak sapwood	"
Hylotrupes bajulus	"	280	100	in pine timber	"
Sirex juvencus	"	600	100	free from wood	"
Uroceras gigas	"	1000	99.9	larch wood 15-20% M.C.	"
Prionoplus reticularis	25	150	100	fumigation chamber	Cross 1991
Arhopalus tristis	"	100	100	"	"
Hylastes ater	"	100	100	"	"
Kalotermes brouni	"	100	100	"	"

Table 8. Toxicity of methyl bromide to wood boring insects as determined by field studies.

Target insect	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Hylotrupes bajulus	?	1344	100	4 cm in fir wood	Hadlington and Campbell 1956
Lyctus sp.	?	1344	100	1 cm in hardwood	"
Cryptotermes brevis	22-27	680	70	3.2 cm thick fir blocks	Bess and Ota 1960
Scolytus multistriatus	≤40	1577	99.7	in elm firewood	Hanula and Berisford 1980
Tropical insects of 5 families	?	3000	100	in hardwood logs	Tang et al. 1985

The laboratory studies of fungitoxicity have been tabulated for comparison purposes and show the general range of susceptibility of target fungi (Tables 9,10). The Oomycetes are generally more susceptible to methyl bromide, while the Basidiomycetes intermediate and the Ascomycetes and imperfect fungi are more resistant. It should be stated that some plant pathogens are much more resistant than indicated in the table. *Alternaria solani* Sorauer spores on glass fiber filter disks were subjected to CxT values as high as 760,000 at low temperature (5° C) and humidities (0-8%). This massive dosage only achieved 50% mortality of this imperfect fungal species (Munnecke et al., 1959). The lowest CxT value reported for these spores was 12,000 for 50% mortality. This was for fumigations performed at 23° C and 49% relative humidity. Mortality was increased for all temperatures at

moderate relative humidities due to increased spore germination under these conditions.

As with insects, temperature also exerts a profound influence on the success of fumigation against pathogenic fungi, probably also due to increased respiration. Munnecke and Bricker (1978) found methyl bromide dosages necessary to kill 90% of the propagules of *Pythium ultimatum* Trow were about 3.8 times greater at 5° C than at 30° C. This is also partially responsible for the difference in CxT values reported for identical species tested by Munnecke et al. (1978) and Ebben et al. (1983). The difference in level of mortality reported was probably also a major factor; differences in laboratory technique were probably another, although concentrations of fumigant used were almost identical.

Although bark is presumed to slow fumigant penetration, the oak logs fumigated in all laboratory and field studies (Table 11) reported here, had the bark on. This probably required higher CxT values than would have otherwise been necessary. However, since the oak wilt fungus grows in the inner bark and not more than 32 mm into the sapwood of an oak log (Jones and Bretz, 1955), fumigant penetration to the center of a log is not necessary. The Dutch elm disease fungus usually only grows in the cambium and outer two annual rings (Stipes and Campana, 1981). This and the high temperatures reached during field fumigations, in addition to the small size of the wood tested, probably account for its relative sensitivity to methyl bromide.

It should be pointed out that while Schmidt et al. (1982) killed the oak wilt fungus in all six chamber fumigations with CxT values from 10,800-12,492, they occasionally found decay fungi present in the sapwood. The

field results that were reported with these laboratory findings isolated the oak wilt fungus from oak logs subjected to CxT products as high as 14,500. This indicated the need to add more gas during the exposure period to maintain a high concentration outside of the logs.

In later studies (Liese and Ruetze, 1985; MacDonald et al., 1985) more fumigant was added after 24 hours to replace the fumigant lost through sorption or leakage, and ensured adequate CxT levels to the depth where the fungus grew. Higher initial concentrations were not tested because 240 mg/l was considered to be the maximum concentration that could be handled safely using conventional tent fumigation techniques (Liese and Ruetze, 1985). This fumigation of 240 mg/l for 72 hours with more gas added at 24 hours to bring the level back to 240 mg/l is referred to the T312 schedule by USDA, APHIS.

The Oregon Department of Agriculture also noted the resistance of stain fungi in green wood to fumigants. In an unpublished letter of March 26, 1993 they reported that core samples of radiata pine logs imported from Chile contained five distinct fungal species. The logs in question had been subjected to the methyl bromide T404 schedule with a CxT product of 1920 mgh/l (80 mg/l for 24 hours with no topping off). One of these species was a vascular wilt fungus (*Leptographium* sp.) similar to the oak wilt or Dutch elm disease fungi (J. Stone, personal communication).

Parameswaran and Ruetze (1985, abstract only) investigated the toxic effect of methyl bromide on the oak wilt fungus. Treated hyphae showed a comprehensive degradation of the protoplast. Both cell walls and organelles, particularly mitochondria, ribosomes and membranous structures were degraded. They summarized the

toxic effect to be the result of overall chemical damage and not the result of interference of a single biochemical process.

Ruetze and Liese (1985) found that methyl bromide fumigation that killed the oak wilt fungus also killed living sapwood cells of oak trees infested with the fungus. They devised a tetrazoliumchloride test to indicate the presence of living parenchyma cells in the sapwood which in turn indicated poorly fumigated or non fumigated logs. Negative reactions due to excessive drying of the logs would also kill the fungus. Hanula and Berisford (1982) found uninfested elm firewood treated with methyl bromide was only weakly attractive to smaller European elm bark beetles (*Scolytus multistriatus* [Marsham]) after treatment, and small broods were produced by the few that did infest post-treatment.

Methyl bromide has also been tested to control root diseases of forest trees and decay fungi in utility poles. Graham (1975) eliminated decay fungi from utility poles in service with the fumigant. Houston and Eno (1969) tested its efficacy against *H. annosum* in roots of three species of pines, and found difficulty in eliminating the fungus in roots larger than 15 cm in diameter. Harvey (unpublished, from Thies and Nelson, 1986) used methyl bromide to eliminate *Phellinus weirii* (Murr.) Gilbn. in 5 cm cubes of infested stump wood placed in soil in plastic containers. Filip and Roth (1977) found it effective in eliminating *Armillaria ostoyae* (Romagn.) Herink from infested ponderosa pine (*Pinus ponderosa* Laws.) stumps in the forest soil. However, the applicability of these studies is limited because in none of them was the dosage of fumigant effectively contained over a specified time period. Thus, CxT values cannot be computed for comparison.

Table 9. Toxicity of methyl bromide to Oomycetes and Basidiomycetes as determined by laboratory studies.

Target Fungi	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Pythium ultimatum	24	469	90	mycelial culture	Munnecke et al. 1978
Phytophthora cinnamomi	24	286	90	chlamydo-spores in soil	"
"	"	485	90	roots in soil	"
"	"	461	90	mycelial culture	"
Armillaria mellea	24	779	90	"	"
Rhizoctonia solani	24	795	90	"	"
"	10	<1911	99	cultures on petri plates	Ebben et al. 1983
Poria carbonica	25	2093	100	"	Ricard 1966

Table 10. Toxicity of methyl bromide to Ascomycetes as determined by laboratory studies.

Target Fungi	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Verticillium albo-atrum	24	1311	90	infested stems	Munnecke et al. 1978
"	"	1390	90	cultures on agar	"
"	10	2688	99	"	Ebben et al. 1983
Phomopsis sclerotioides	10	>2688	99	"	"
Fusarium oxysporum	10	2688	99	"	"
Ceratocystis fagacearum	23	72,000	100	oak log sections in chamber	Partridge 1961
"	5	10,800	100	"	Schmidt et al. 1982
"	"	5600	100	"	"
"	"	3230	69	"	"
"	"	3792	67	"	"
"	"	16,128	100	"	Schmidt 1983
"	0	14,976	100	"	"
"	-5	15,696	100	"	"
"	-5	15,648	98	"	"
"	-10	21,188	79	"	"
"	0	1920	100	cultures on agar	Liese and Reutze 1985
"	-10	3960	0	"	"

Table 11. Toxicity of methyl bromide to wood inhabiting fungi as determined by field studies.

Target fungi	Temp. °C	CxT mgh/l	Mortality %	Conditions	Author
Ceratocystis fagacearum	3-33	44,856	100	in 250 cm oak logs	Jones 1963
"	10-16	9360	92	"	Schmidt et al. 1982
"	"	14,500	99.6	"	"
"	0-6	15,031	100	in 530 cm oak logs	Liese and Reutze 1985
"	3-6	11,520	100	in 500 cm oak logs	MacDonald et al. 1985
"	"	17,280	100	"	"
Ceratocystis ulmi	$\leq$40	1536	100	in elm firewood	Berisford et al. 1980
"	"	"	92	"	"

III OBJECTIVE OF THE STUDY

The objective of this study was to derive minimum concentration time values for methyl bromide necessary to kill fungi representative of those likely to be inhabiting Dahurian larch wood, as a guide to subsequent field trials.

IV MATERIALS AND METHODS

Three sets of fumigation tests were performed. The preliminary tests will be described first. Next, leak tests will be described. Leak tests were designed to estimate the rate of methyl bromide leakage during preliminary fumigations. These tests also evaluated the efficacy of improved fumigation techniques. Refined experiments incorporating these improved techniques will be described last.

A. Preliminary Tests

1. Fungal Species Tested

The species tested were chosen from lists in the USDA, Forest Service Pest Risk Assessment (1991) and Rozhkov (1970). Criteria for selection were the probability of host association, transport potential, survival, establishment, colonization and potential losses associated with introduction. Several fungal pathogens and saprophytes were tested to ensure methyl bromide effectiveness against as many species as time allowed. The fifteen fungal isolates tested in preliminary tests were:

Fomitopsis cajanderi (Karst.) Kotl. et Pouz.
FP 10796
Fomitopsis officinalis (Vill.: Fr.) Bond et Singer
FRL OSU
Fomitopsis pinicola (Schwartz: Fr.) Karst.
RLG 5047
Heterobasidion annosum (Fr.) Bref.

FRL OSU
Heterobasidion annosum (Fr.) Bref.
PPL OSU
Lachnellula wilkommii (Hart.) Den.
LC-30 UM
Laetiporous sulfureus (Bull.:Fr.)Bond et Sing.
105530-SP
Leptographium wageneri (Kendr.) Wingf.
PPL OSU
Oligoporous obductus (Berk.) Gilbn. & Ryv.
=*Postia obducta* (Berk.) Larsen et Lomb.
L-14124-SP
Perenniporia subacidia (Pk.) Donk
=*Poria subacidia* (Pk.) Sacc.
FSL OSU
Phaeolus schweinitzii (Fr.) Pat.
FP 14854-5
Phellinus pini (Thore: Fr.) A. Ames
PROL6 PPL OSU
Phellinus weirii (Murr.) Gilbn.
FP 91601
Trametes versicolor (L.: Fr.) Pilat.
FP 105
Trichaptum abietinus (Dicks.: Fr.) Ryv.
=*Hirschoporous abietinus* (Dicks.: Fr.) Donk
L-15831-SP

While *Leptographium wageneri* is a native fungus and not on the previously cited lists, it was chosen to represent a typical vascular stain fungus.

2. Wood Samples

Kiln dried Dahurian larch wood samples from the Lake Baykal region of Russia (Olga Krankina, personal communication) were obtained from a cooperator and cut into one centimeter cubes. The cubes were cut from several boards, and were randomly mixed to control inherent variations in wood permeability. Most of the cubes were

composed entirely of heartwood. Cubes with gross defects were omitted.

3. Fungal Inoculation

Each test fungus was grown on 2% liquid malt extract for 5 to 15 days at room temperature in stationary culture. The liquid inoculum was mixed in a Waring (R) blender and poured over 55 Dahurian larch wood cubes in a plastic bag with a gas permeable patch using the malt amended vermiculite burial method described by Sexton et al. (1993/1994). The bags were incubated for at least six weeks at 27° C to permit complete wood colonization. This procedure saturated wood cubes to relatively uniformly high moisture contents during incubation to minimize methyl bromide penetration and simulate the interior of a wet, partially decayed log. At least eight bags were prepared for each fungal isolate to ensure adequate numbers of cubes should some become contaminated.

Bags inoculated with one of fifteen target fungal isolates were randomly designated in one of three replicates and one of two temperature regimes (0° C and 18° C). The third 0° C replicate was not conducted because the first two 0° C treatments were obviously ineffective. The order of fumigations with respect to initial concentration (0 mg/l, 79 mg/l, and 237 mg/l) and time (8, 24, and 72 hours) was also randomized so that blocks from each bag would be tested for all nine CxT combinations.

After the incubation period, the gas permeable bags were opened one at a time in a laminar flow hood, and the inoculated cubes were removed from the bag one at a time. The vermiculite was scraped off into a waste beaker, and the

cubes were surface sterilized by briefly flaming. Five cubes from the same bag were then placed in one fumigation chamber. The bag was then resealed with an impulse sealer. This was repeated for all fifteen test isolates.

4. Fumigation

Fumigation chambers consisted of nominal 120 ml squat glass jars (actual average volume 133 ml) with Teflon (R) lined lids. Male threads of the glass jars were wrapped with Teflon tape, and female threads of the lids were sealed with silicone grease to minimize fumigant loss. Chambers and lids were sprayed with a light mist of 95% ethanol for surface sterilization, and allowed to dry. Five cubes were placed on a nylon screen inside the jar supported by a four legged stainless steel stand with clearance to accommodate a 25.4 mm long by 7.94 mm diameter stirring magnet to enhance fumigant mixing in the jar. The lids were then placed loosely on each jar to reduce the chance of contamination. Next, the chambers were chilled at -10° C in a freezer for at least 20 minutes.

Neat methyl bromide was stored in a refrigerator at 0° C, and dispensed as liquid from a 500 g lecture bottle through a 22 gauge needle attached to the second of two shut off valves with a 25 mm section of 6 mm inner diameter Teflon tubing. The tubing was held tightly in place with nylon tubing clips at both ends. The end attached to the needle required Teflon tape to form a tight seal. The lecture bottle was turned and held in a horizontal position in a laboratory stand as the two valves were opened and liquid methyl bromide was dripped into a 1 ml glass vial. When the

vial was about half filled, the second shut off valve was closed, and the vial was sealed with a Teflon lined lid. The vial was placed in an ice filled basin, and the space between the two valves was purged before shutting off both valves and returning the lecture bottle to the refrigerator for storage. All methyl bromide storage and application procedures were performed within a laboratory fume hood.

Fumigation chambers were randomly removed from the freezer and placed in the ice filled basin. The prescribed amount of methyl bromide was drawn from the vial with a gas tight syringe primed with diethyl ether. Occasionally the syringe required a very small amount of silicone grease applied to the tip of the plunger to maintain a seal. Accuracy of 25 µl syringes used for methyl bromide application was $\pm$ 2 mg/l. Methyl bromide was introduced into the chamber by first removing the unscrewed lid atop the jar. As the vial and syringe were held less than 5 cm directly over the open jar, the syringe was drawn from the vial, and dispensed against the glass at the bottom of the jar. Thus, any fumigant that spilled from the syringe fell into the chilled jar. The lid was quickly sealed, and the next fumigation chamber was removed from the freezer. Controls were sealed as they were removed from the freezer. The process was repeated until all fifteen chambers in a run received the same amount of liquid methyl bromide. The sealed jars were randomly placed on a 15 station stir plate; the rheostat of the stir plate was adjusted to about 200 revolutions per minute for all fumigations.

After the allotted time had expired, the stir plate was turned off and the chambers were opened. One cube per stir plate was chosen at random to determine the level of methyl bromide by immediately dropping the cube into a glass vial

containing 5 ml of diethyl ether. The glass vial was then sealed in the same manner as the fumigation chambers and stored for at least 48 hours. Methyl bromide concentrations were determined via gas chromatography as described below. One cube was also chosen at random for moisture content determination. The cube was immediately weighed, dried for at least eight hours in an oven at 90° C and reweighed. The remainder of the cubes were aerated under the fume hood in their chambers with the lids loosely ajar for at least 24 hours.

5. Fungal Survival Assessment

The chambers were then moved to the laminar flow hood. The cubes were removed with forceps, placed on an aluminum block, and quartered with a razor blade. Each quarter was then flamed and placed on 2% malt extract agar amended with 10 ppm benomyl to minimize growth of contaminating fungi. Blocks colonized by *L. wageneri* and *L. wilkommii* were plated on unamended malt extract agar, since benomyl inhibits the growth of this fungus.

The plates were then observed for at least fourteen days for evidence of growth of the target fungus. Often contaminating benomyl tolerant molds, basidiomycetes or bacteria were present. Difficult identifications were referred to Camille Freitag, a mycologist experienced with identification of wood decaying fungi. The presence or absence of target fungal growth or confounding contamination was recorded. The percentage of cube quarter sections that formed colonies on agar was used to measure fungal survival for each concentration, time and temperature combination

replicate. This was used to assess the efficacy of treatment. Contaminated quarter sections were recorded but not used to calculate survival percentages. Jars with more than 50% of cube quarter sections contaminated were not included in data analysis.

6. Methyl Bromide Measurements

All methyl bromide concentrations were determined with a Varian model 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. The glass column was 1.524 meters long with a 2 mm inner diameter and a 6.35 mm outer diameter. It was packed with 20% SP 2100 and 0.1% Carbowax 1500, on a 100/120 Supelcoport mesh coating. The column temperature was held at 40° C for 1 minute, by which time the methyl bromide peak had appeared. The column temperature was then raised at a rate of 80° C per minute to 100 C and held at that temperature for one minute to purge the column of extraneous impurities. The detector and injector temperatures were held at 240° C and 150° C, respectively. Nitrogen was used as a carrier gas at a flow rate of 54 ml per minute.

Methyl bromide standards were made by serial dilutions of 2.0 M methyl bromide in diethyl ether. Standards were stored in glass tubes with male threads wrapped in Teflon tape and tightly capped with Teflon lined lids at 0° C. Fresh standards were made at least every two weeks. Comparison of peaks from fresh and older standards revealed less than 2% difference in peak heights.

B. Leak Tests

Leak tests were undertaken to determine the stability of methyl bromide dosages in jars at the end of the fumigation period. Also, it was thought that side by side fumigations with initial and improved methods might provide a common index for comparison of results.

It was surmised that injection ports installed into the jar lids would be an efficient means of both adding initial methyl bromide dosages and sampling final methyl bromide concentrations in air. Injection ports consisting of one 6.35 mm X 9.53 mm brass compression fitting were installed in the lids of half of the fumigation chambers. The 6.35 mm end was tapped while hot into a 6.35 mm hole drilled into the lid. The joint was sealed by Fix All,(NCH Corporation, Irving, Texas), a very flexible cohesive resin sealant. The threads of the 9.53 mm end were wrapped in Teflon tape and a 7.70 mm septum was snugly screwed down to the open end with the 9.53 mm nut. The other half of the fumigation chambers were tested without injection ports as in the preliminary fumigation tests.

Both types of fumigation chambers were tested side by side in a systematic experiment, with three replicates of all three concentrations conducted as one run at a particular fumigation time. Thus, a run consisted of eighteen separate fumigations, fifteen chambers on the fifteen station stir plate and three chambers on three one station stir plates. Unported jars received five cubes as before, while ported jars received six. The extra cube was added to increase the sampling intensity of methyl bromide in wood at the end of fumigation in the refined studies. It was included in the leak tests for comparison purposes. Methyl bromide

concentrations in each cube of all jars were measured in order to sample these levels completely. A moisture content sample cube was taken from each gas permeable plastic bag and measured as in preliminary studies. This was done as the other cubes were being placed into fumigation jars.

At the end of fumigation time, two 10 µl air samples were taken from each ported jar through the Teflon septa. Air samples were also taken from the unported jars of the 8 hour fumigation as the lids were opened. The results were questionable, however, due to presumed mixing with the outside air. Jars were opened, and all blocks were dropped in separate glass vials with 5 ml of diethyl ether. Both samples were analyzed for methyl bromide content via gas chromatography as described previously. By adding the amount of methyl bromide in the wood to the amount in the air of the jar at the end of the fumigation time, the total amount in the jar at the end of the fumigation time was calculated and the difference between this amount and the initial amount added was the amount that leaked from the jar. Chemical leakage and sorption was assumed to be linear over fumigation time from the initial amount added to the jar when computing cumulative CxT values as described by Monro (1969).

C. Refined Fumigation Experiments

The refined fumigation experiments tested only five fungal species considered to be the most important to determine lethal CxT values for from a plant quarantine standpoint. The fungal isolates tested were:

Armillaria ostoyae (Romang.) Herink
AO-4 FSL OSU
Heterobasidion annosum (Fr.) Bref.
FRL OSU
Lachnellula wilkommii (Hart.) Den.
LC-30 UM
Leptographium wageneri (Kendr.) Wingf.
PPL OSU
Phellinus weirii (Murr.) Gilbn.
FP 91601

Isolates were cultured by the same method as before but care was taken to ensure that all wood cubes were composed entirely of heartwood to minimize variability of wood permeability.

Each species was tested separately at a particular fumigation time with six replicates of each at the three methyl bromide concentrations. Eighteen total fumigation chambers were tested during one run as in the leak tests. Thus, cubes from each of two gas permeable plastic bags were used in three consecutive replicates at each concentration. This systematic design increased the number of fumigations that could be performed in the allotted time, and made planning routine tasks easier.

In these experiments methyl bromide was applied into jars as in preliminary experiments. Care was taken to only apply a thin coat of silicon grease to female threads of the jars and diligently apply Teflon tape to the male threads as smoothly as possible. Ported chambers were used, but the port was only used for air sampling. Two air samples were taken as before and the derived concentrations were averaged. After the jars were opened two cubes were immediately dropped into separate vials containing 6 ml of diethyl ether. This submerged the cube more completely than 5 ml of solvent. Care was taken to allow between 2 and 4 days for solvent

extraction before sampling methyl bromide levels via gas chromatography. These two samples were also averaged after their concentrations were derived with gas chromatography.

The four remaining cubes were aerated, quartered and plated as in the preliminary studies. Selective media was prepared for *L. wilkommii* as described by Hiley (1919). This required amending 3% malt extract agar with beef extract and citric acid. Selective media was also prepared for *A. ostoyae* by adding dextrose and bacto-peptone to 4% malt extract agar as described by Shaw and Roth (1976). These media were used for both culturing of cubes in bags (without agar), and plating cube quarter sections after fumigation.

D. Data Analysis

For preliminary and leak tests, data was merely tabulated. For the refined tests gas leakage, sorption, and final concentration were analyzed graphically to estimate fumigant movement over time, while consistency of methyl bromide dosages was tabulated. For each species individually and all species collectively, average concentrations were graphed with respect to treatment time with fumigations plotted as either successful or unsuccessful to determine the relative importance of these two factors. For each species individually and all species collectively, pecent mortality was graphed with respect to increasing CxT value. Also, the CxT values and resulting mortalities from refined tests were logarithmically transformed. Controls were not included in transformed analysis, and 0.001 was added to all replicates with no mortality since the log of zero is undefined. Transformed data was subjected to a one-way analysis of

variance to determine the significance of CxT on mortality. Due to limited sample sizes, data from all species were analyzed collectively.

V RESULTS AND DISCUSSION

A. Preliminary Tests

The preliminary tests were plagued by both contamination of samples, particularly by molds, and insufficient methyl bromide sampling. As a result, data are only presented for fungal species which had at least two uncontaminated control replicates at each fumigation time (Tables 12 and 13).

Inadequate methyl bromide sampling, particularly the lack of air samples at the end of fumigation made calculation of CxT values impossible because of unknown gas leakage. Wide variations in methyl bromide levels were detected in wood cubes at the end of treatments with common initial concentrations and fumigation times. This indicated a need to determine variations in methyl bromide concentrations, both in the air of the jars and within cubes at the end of the fumigation period.

Another important shortcoming of the preliminary tests was that six (10.3%) cubes sampled from non-control jars contained only traces of methyl bromide at the end of fumigation. Half of these were in 0° C fumigations and probably reflect the inability of the fumigant to volatilize and penetrate the wood. However, this indicated excessive leakage from some jars, and led to an attempt to identify which replicates to exclude from data analysis in subsequent tests. In the preliminary tests, fumigant leakage was a persistent but randomized problem.

Another problem with the preliminary tests was solvent leakage. Solvent was lost from 14 vials (24.1%) containing wood samples, before adequate sampling techniques were

developed. Data from vials that were sampled were not considered reliable because many of them were stored too long and lost noticeable amounts of solvent. Due to the inadequacies listed, none of the methyl bromide assays from these tests are reported.

1. Frozen Replicates

Results of experiments conducted at 0° C demonstrated the ineffectiveness of methyl bromide using the techniques previously described, against the eight fungal species for which usable data were recorded. This lack of efficacy probably reflects the inability of methyl bromide to adequately volatilize. Methyl bromide has a vapor pressure of 690 mm Hg at 0° C (Munro, 1969). Thus, most of the chemical would remain in the liquid state where it would be unable to penetrate the wood cubes and kill the fungi. Of the 16 non-control wood samples tested for methyl bromide levels, 13 contained quantities of the chemical that should have been sufficient to kill target fungi (324-3569 mg/l) (Munnecke et al., 1978). However, only five of these analyses corresponded to uncontaminated replicates making it difficult to draw conclusions.

The few replicates exposed to fumigant at 0° C that showed any level of fungal control probably resulted from fumigant spilling out of the syringe directly onto the wood cubes instead of against the side of the glass jar, as intended. In most cases this was avoided and the bulk of the fumigant probably remained near the bottom of the jar until the fumigation was ended and the jars were allowed to aerate at room temperature. The vigorous action of the stirring

magnet could also have thrown liquid or partially volatilized methyl bromide onto the cubes. The chemical that did reach the saturated (moisture content of the samples varied from 91-228%), frozen wood probably did not penetrate deeply enough to kill the fungi.

Table 12. Percent mortality of fungal species exposed to nine concentration and time combinations of methyl bromide at 0°C.

Conc.(mg/l)		0			79			237		
Time (hours)		8	24	72	8	24	72	8	24	72
Target CxT (mgh/l)		0	0	0	632	1896	5688	1896	5688	17064
Species	Rep.									
Fomitopsis cajanderi	1	0	0	0	0	0	37	0	0	17
	2	0	0	5	0	0	0	0	0	0
Fomitopsis officinalis	1	0	0	0	0	0	0	0	0	6
	2	0	0	0	0	0	0	0	63	80
Fomitopsis pinicola	1	0	0	11	0	0	6	5	61	55
	2	0	0	0	0	0	0	0	40	100
Laetiporous sulfureus	1	0	0	0	0	0	0	91	0	C
	2	0	0	0	0	0	0	100	0	5
Leptographium wageneri	1	0	0	0	0	0	0	0	28	C
	2	0	0	0	0	0	0	0	0	0
Perenniporia subacidia	1	0	5	15	0	0	95	7	0	30
	2	0	0	0	6	0	0	0	0	6
Phaeolus schweinitzii	1	0	5	15	5	0	0	82	0	C
	2	6	0	0	0	0	0	0	0	0
Trametes versicolor	1	0	0	0	0	0	0	0	0	18
	2	0	0	0	0	0	0	0	0	0

C = > 50% of cube quarter sections were contaminated

This study stands in contrast to the cold temperature laboratory fumigations to destroy the oak wilt fungus. Liese et al. (1981) achieved fungicidal dosages at least 4 cm deep in oak logs during fumigations conducted at -3° C. Schmidt (1983) obtained complete control of the same fungus in red oak log sections with fumigations conducted at 0° C and CxT values as low as 14,976 mgh/l. Three fumigations conducted at -5° C with CxT values between 14,592 and 15,696 reduced frequency of fungal isolation from 30% to less than 1%. Fumigations conducted at -10° C were ineffective, even when gas concentration or exposure durations were increased by 50%. However, all fumigations in his study reported here were vented at 0° C for four days to allow the fumigant to evaporate while below its boiling point. Liese and Reutze (1985) killed 100% of isolates of the oak wilt fungus in petri plates during fumigations with CxT values of 1920 and 2304 at 0° C. However, fumigations conducted at -10° C were ineffective, even with CxT products as high as 3960.

Many of the discrepancies between the current study and previous ones are probably due to the different methodology for applying methyl bromide. In all previous studies mentioned, liquid methyl bromide was allowed to volatilize by applying it into a fumigation chamber through copper tubing in water at 50° C. This would have allowed gaseous methyl bromide to adhere to the entire surface of the wood or agar and penetrate to some extent before condensing.

2. Room Temperature Replicates

Bioassays of the nine fungal species with at least two replicates of uncontaminated controls showed methyl bromide fumigation at room temperature to be much more effective than at 0° C. The difference is probably due to the increased vapor pressure of methyl bromide at 18° C of 1313 mm Hg (Munro, 1969), which is nearly double the vapor pressure at 0° C. Increased fungal respiration at the higher temperature probably also led to increased fumigant uptake by the target fungi, and consequently increased mortality.

No fungal survival was noted with the highest CxT product (237 mg/l for 72 hours). The 24 and 8 hour treatments with the same concentration caused about equal mortality, but were not as effective as longer exposures. The inability to accurately predict leakage makes actual CxT products impossible to calculate, but the complete mortality associated with the highest nominal CxT indicates that it was less than 17,064 mgh/l.

Fumigations with the lower methyl bromide concentration (79 mg/l) were not as effective as those with the higher concentration regardless of exposure time. Fumigations at 79 mg/l for 72 hours were generally less effective than those with initial concentrations of 237 mg/l held for eight hours, even though they had only one-third the nominal CxT product. This effect can be at least partially explained by the higher diffusion gradient with the higher initial concentration saturating the small wood cubes and reaching equilibrium more quickly than the lower initial concentration. These tests used small pieces of wood, making long distance diffusion less relevant than it would be for field fumigation of large logs.

The very small sample sizes and the inability to accurately calculate CxT products make it difficult to draw conclusions concerning relative tolerance of the fungi tested to methyl bromide. However, *L. sulfureus* appeared to be quite tolerant. *H. annosum*, *F. pinicola* and *P. schweinitzii* were more sensitive, while the other species were intermediate in susceptibility to methyl bromide.

Table 13. Percent mortality of fungal species exposed to nine concentration and time combinations of methyl bromide at 18°C.

Conc. (mg/l)		0			79			237		
Time (hours)		8	24	72	8	24	72	8	24	72
Target CxT(mgh/l)		0	0	0	632	1896	5688	1896	5688	17064
Species	Rep.									
Fomitopsis cajanderi	1	0	0	0	15	0	100	100	100	100
	2	C	C	C	C	C	100	100	100	100
	3	0	0	0	0	0	0	0	100	100
Fomitopsis pinicola	1	10	0	0	95	100	100	100	100	100
	2	0	0	0	0	C	100	100	100	100
	3	0	0	0	0	0	0	100	100	100
Heterobasidion annosum	1	50	19	C	100	100	100	100	100	100
	2	0	0	0	C	0	10	100	100	100
	3	C	6	10	10	C	36	C	100	100
Laetiporous sulfureus	1	20	0	0	20	0	15	100	100	100
	2	0	0	0	0	C	0	100	7	100
	3	16	0	0	0	0	0	25	90	100
Leptographium wageneri	1	0	6	0	0	0	100	100	100	100
	2	C	C	C	C	C	C	100	100	100
	3	0	O	0	O	O	0	100	0	100
Perenniporia subacidia	1	10	10	20	55	100	100	58	100	100
	2	0	10	0	0	C	0	100	100	100
	3	0	0	0	0	0	100	15	6	100
Phaeolus schweinitzii	1	0	0	0	0	C	100	100	100	100
	2	0	C	C	0	C	C	100	C	100
	3	C	0	0	C	100	0	100	100	100
Phellinus weirii	1	30	6	0	12	44	100	100	100	100
	2	0	17	6	0	0	100	100	100	100
	3	15	0	0	0	C	7	100	50	100
Trametes versicolor	1	C	C	C	0	0	100	100	100	100
	2	0	0	0	0	0	0	C	100	100
	3	0	0	0	0	0	0	0	100	100

C = > 50% of cube quarter sections were contaminated

B. Leak Tests

Results of the leak tests are displayed in Table 14. Many ported, non-control jars in these tests contained no trace of methyl bromide at the end of fumigation. In these instances, it is likely that the liquid methyl bromide drained out of the syringe before the needle pierced the septum. Thus, no methyl bromide was injected into these jars, since even jars with excessive leakage always contained detectable traces of chemical at the conclusion of a test. Other ported jars had only traces of methyl bromide at the end of fumigation period, indicating that only a partial dose was injected through the septa and/or these jars leaked excessively. These results suggest that application of methyl bromide through the septa was not effective.

Results of the unported jars were more favorable; however, none of the three replicates of 79 mg/l for 24 hours contained traces of methyl bromide. It was deduced that this was caused by applicator error and that no methyl bromide was injected into these jars, most likely they were overlooked completely and treated as controls. The few air samples that were taken in unported jars cannot be considered reliable, because the lids needed to be removed for sampling, resulting in some mixing with outside air.

Table 14. Results of leak tests. Jars contained wood blocks colonized by *T. abietinus* and selected levels of methyl bromide.

		Methyl Bromide Concentration (mg/l)[a]					
Initial conc.		72 hour		24 hour		8 hour	
	Rep.	Wood	Air	Wood	Air	Wood	Air
ported 237 mg/l 6 cubes in each jar	1	1278(40)	36	386(35)	65	T	T
	2	T	0	T	T	T	T
	3	T	0	T	0	0	T
unported 237 mg/l 5 cubes in each jar	1	1501(150)	N.S.	T	N.S.	T	0
	2	1317(71)	N.S.	T	N.S.	1146(68)	31
	3	755(44)	N.S.	1465(14)	N.S.	1018(23)	34
ported 79 mg/l 6 cubes in each jar	1	231(7)	6	0	0	T	T
	2	0	T	0	0	120(5)	T
	3	242(27)	3	0	0	280(10)	43
unported 79 mg/l 5 cubes in each jar	1	562(66)	N.S.	0	N.S.	295(10)	19
	2	537(65)	N.S.	0	N.S.	287(10)	41
	3	37(10)	N.S.	0	N.S.	156(8)	9
ported 0 mg/l 6 cubes in each jar	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
unported 0 mg/l 5 cubes in each jar	1	0	N.S.	0	N.S.	0	0
	2	0	N.S.	0	N.S.	0	0
	3	0	N.S.	0	N.S.	0	0

[a]N.S. = No sample taken, T = Trace of methyl bromide detected. Wood values represent means, while figures in parentheses represent one standard deviation.

Jars that received the proper dosage showed fairly uniform methyl bromide levels between wood cubes. However, variability between jars was quite high. The reasons for this were probably due to the differences in the seals of individual jars. Factors such as the amount of torque used to screw down the lid, the smoothness with which the Teflon tape was applied to the male threads, and the amount and uniformity of the silicon grease layer applied to the female threads were probably the main sources of variation. Unfortunately, these factors were not standardized. However, as more tests were conducted the uniformity of the technique increased.

C. Refined Tests

All data collected for the refined tests are displayed in Appendix B. Results of analysis of variance of logarithmically transformed CxT values on logarithmically transformed mortality are displayed in Appendix C. There was a very highly significant ($p = 0.0001$) effect of CxT on survival of the fungi in the wood cubes. Data for *P. weirii* is reported in the Appendix but was not included in data analysis because the controls for this fungus were completely contaminated. Although 31 replicates of the other four species were rejected due to contamination and/or excessive leakage, a series of fumigations (replicate numbers 7-12 for each species and treatment) were conducted to replace the faulty replicates. Therefore, all treatment combinations for each of the four species analyzed had at least three and usually six, and in two instances, as many as eleven replicates for which usable data were recorded.

1. Consistancy of Dosages

Data for consistancy of dosages for nominal treatments are displayed in Table 15. There was wide variation within nominal non-control treatments. Because of this variation, each replicate had a unique CxT product and nominal treatments were considered as treatment groups.

Variation was probably primarily due to differences in leakage between jars which will be discussed in more detail later. Other possible sources of variation included differences in sorption due to variations in wood anatomy and moisture content. Sorption to the glass jars and Teflon lids of the containers must also be considered, although these factors were probably fairly uniform between jars.

Although variation of the initial amount of methyl bromide applied cannot be known with certainty, the smallest graduations of the syringes used to dispense the chemical correspond to approximately 2 mg/l. Since the liquid methyl bromide could be clearly seen in the syringe as it was being drawn into the syringe, and the syringe was held directly over the open jar as it was removed from the vial, any chemical that spilled from the vial would have fallen into the chilled jar and remained liquid. For these reasons it is believed that the standard deviation of the initial dosages was approximately 2 mg/l due primarily to the limitations of the syringe.

As can be readily discerned, wood selectively sorbs methyl bromide from the air. Most of the concentrations measured in wood were 5 to 15 times those of air. Sorption of methyl bromide by wood will be discussed in more detail below.

Table 15. Consistency of methyl bromide dosages. Values are concentrations for the air of glass jars and wood cubes therein colonized by various fungi at the end of fumigation time. Wood and air values represent means, while figures in parentheses represent one standard deviation.

Target species	Initial Conc. (mg/l)	72 hour (mg/l)		24 hour (mg/l)		8 hour (mg/l)	
		wood	air	wood	air	wood	air
Armillaria ostoyae	237	817 (343)	31 (8)	1016 (412)	37 (11)	990 (326)	77 (7)
Heterobasidion annosum	237	159 (96)	18 (11)	381 (310)	77 (45)	875 (350)	60 (14)
Lachnellula wilkommii	237	1000 (463)	24 (7)	402 (152)	10 (5)	1094 (289)	62 (8)
Leptographium wageneri	237	921 (48)	81 (1)	1206 (353)	75 (7)	873 (293)	74 (13)
Armillaria ostoyae	79	401 (47)	30 (5)	326 (148)	30 (8)	353 (155)	49 (14)
Heterobasidion annosum	79	74 (43)	9 (2)	74 (43)	14 (10)	310 (138)	30 (16)
Lachnellula wilkommii	79	245 (184)	15 (10)	180 (18)	9 (2)	388 (82)	14 (5)
Leptographium wageneri	79	302 (93)	55 (6)	382 (49)	54 (3)	417 (37)	63 (2)
Armillaria ostoyae	0	0	0	0	0	0	0
Heterobasidion annosum	0	0	0	0	0	0	0
Lachnellula wilkommii	0	0	0	0	0	0	0
Leptographium wageneri	0	0	0	0	0	0	0

2. Fumigant Leakage

Due to the nature of the fumigation techniques used, gas concentrations did not remain constant over the exposure period. Many previous laboratory studies employed large heavily constructed fumigation chambers or continuous flow manifold apparatus. These devices provided for relatively constant monitoring and adjustment of fumigant levels, but required considerably more expense in the case of heavily constructed chambers or operational expertise in the case of a continuous flow manifold apparatus.

Therefore, the results reported herein are not directly comparable to most of the laboratory studies previously listed. However, since initial and final concentrations of methyl bromide were closely monitored in both the wood and air for fumigations of three durations it is possible assess the relative toxicity of methyl bromide to the test species. Indirect comparisons with other laboratory studies can be made after considering the differences in fumigation techniques. However, comparisons with field fumigations are possibly more appropriate than with previously mentioned laboratory studies because this study more closely modeled the unsteady state of fumigant pressure during field fumigations.

As can be seen from Figures 1 and 2, a large percentage of the initial dosage was lost due to leakage in most fumigations, particularly in the first eight hours in jars with higher initial concentrations. The average concentration also rapidly declined for eight hour fumigations at the lower initial concentration, but several replicates lost less than 10%, producing a higher overall

average. Leakage rates lessened over time at both initial concentrations, becoming almost asymptotic by 72 hours.

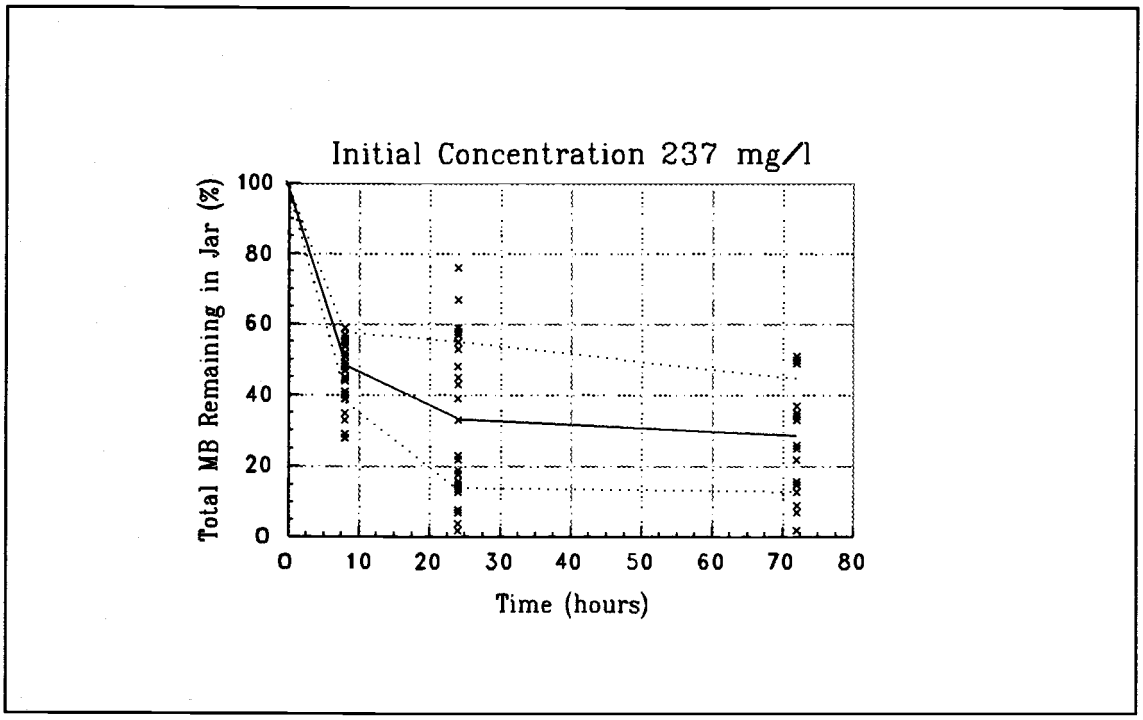

Figure 1. Methyl bromide leakage from jars with initial concentrations of 237 mg/l. Solid lines indicate average concentration over time; dotted line indicates one standard deviation.

The replicates with the higher initial concentration held for eight hours had the least variability of all the treatments tested. This was probably because leakage from the jars was occurring at the maximum rates the gaps in the seals would allow. As fumigant pressures of individual jars decreased, relative differences in the sizes of these gaps between jars probably became more pronounced. Variability of

total methyl bromide in jars remained quite constant from 24 to 72 hours for fumigations with the higher initial concentration. This probably reflects the dampening effect of the fumigant sorbed by the wood being slowly released, counteracting the tendency for the decrease in pressure from the jars to increase variability.

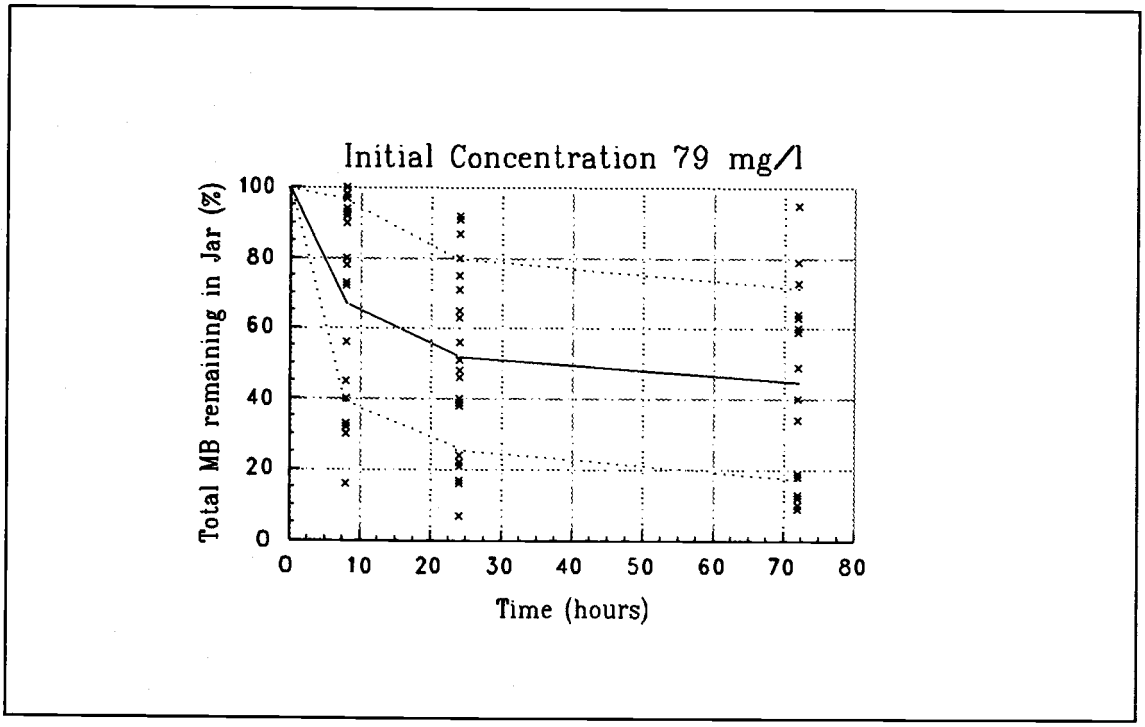

Figure 2. Methyl bromide leakage from jars with initial concentrations of 79 mg/l. Solid lines indicate average rate of leakage over time; dotted line indicates one standard deviation.

The lower pressures resulting from the lower initial concentration also resulted in lower average rates of leakage, and higher relative variability. Variability of the

total amount of fumigant remaining in jars at the end of fumigation was similar for all three fumigation times. Due to lower pressures, some jars with tight seals held most of the fumigant for long periods of time. Conversely, little of the initial dosage was held in jars with poor seals.

With both concentrations, the constant stirring of a 25.4 mm magnet throughout fumigation was unnecessary and probably caused much of the leakage. A smaller stirring magnet would have sufficed.

3. Sorption of Methyl Bromide by Wood Cubes

Sorption of methyl bromide by wet, partially decayed wood cubes in different treatments is shown in Figures 3 and 4. Concentrations were quite variable for all treatments, probably due to factors such as wood anatomy, moisture content and leakage. However, as was the case with fumigant leakage, the eight hour treatments were less variable than the longer treatments. Evidently, both leakage and sorption acted as strong sinks for fumigant. The sorbed gas was only slowly released, as shown by the gradual declines in concentrations from eight to 24 hours, and stable levels from 24 to 72 hours. The gradual increase of the average sorbed gas in wood from 24 to 72 hours, shown at the higher initial concentration, is probably an anomaly that would likely not be present with larger sample sizes. By 72 hours, distinct clusters of final concentrations well above and well below the mean were apparent for both graphs. This probably reflects the integrity of the seal.

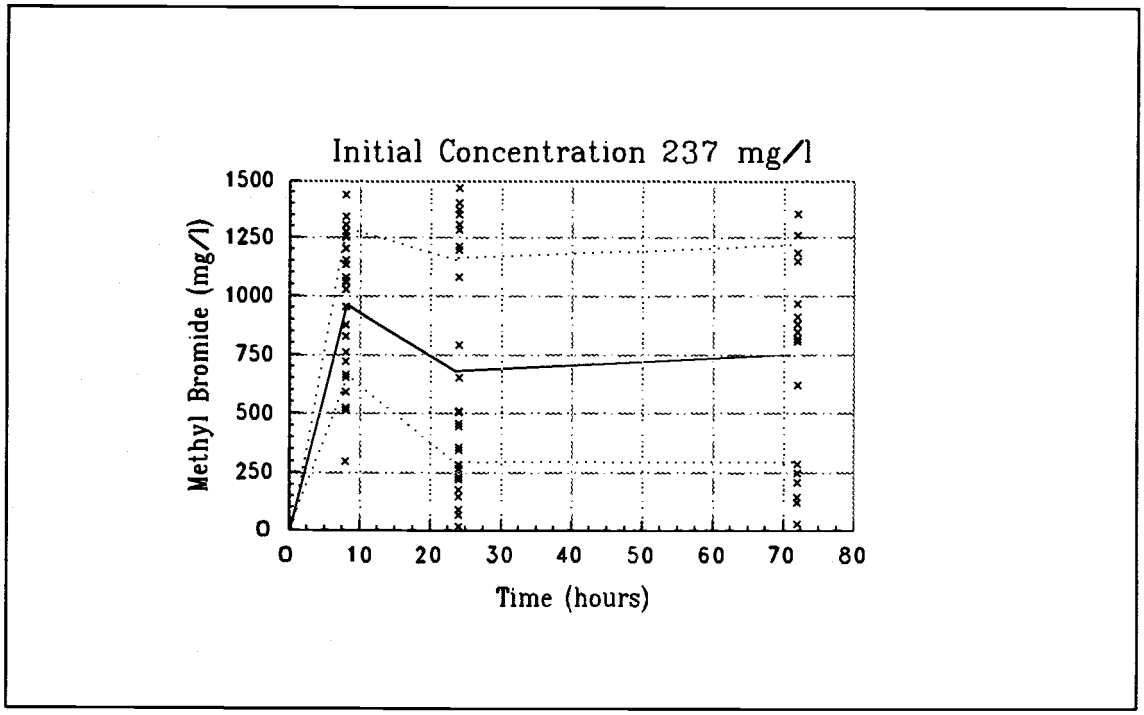

Figure 3. Final concentrations of methyl bromide in wood with initial concentrations of 237 mg/l. Solid line represents mean; dotted lines represent one standard deviation.

Wood moisture contents in blocks, from each inoculation bag were less variable than in the preliminary tests, ranging from 104-148%. This probably reflects increased uniformity of inoculation techniques with increased experience. These relatively high moisture contents may have impeded fumigant penetration by acting as a physical barrier and by directly sorbing the fumigant.

Average methyl bromide concentrations in the wood were 2.6, 2.3 and 3.5 times greater in the higher initial concentrations at 8, 24 and 72 hours than in the lower

initial concentrations, respectively. The selective sorption of methyl bromide by the wood averaged 22 times that of the air for the higher initial concentration at 72 hours. All fumigations with the lower initial concentration sorbed, on average only 9 times as much methyl bromide as the surrounding air. Methyl bromide concentrations in wood with the higher initial concentration averaged about 14 and 12 times as much as the surrounding air for 8 and 24 hour fumigations, respectively. The large concentration sorbed and held over time by the highest treatment combination probably accounts for its efficacy as a fungicidal treatment.

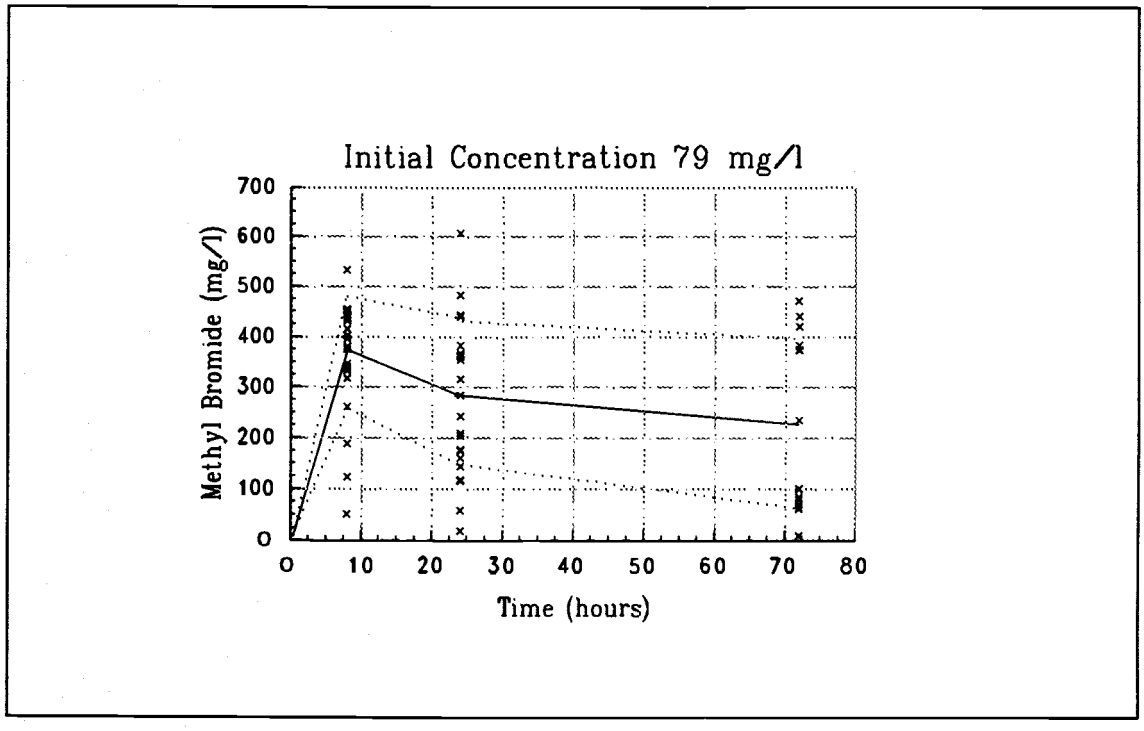

Figure 4. Final concentrations of methyl bromide in wood with initial concentrations of 79 mg/l. Solid line represents mean concentrations; dotted lines represent one standard deviation.

4. Final Fumigant Concentrations of Air in Jars

Final methyl bromide concentrations in the air are shown in Figures 5 and 6 for fumigations with initial average concentrations of 79 mg/l and 237 mg/l, respectively. As can be discerned from the graphs, initial methyl bromide air concentrations decreased more rapidly at the higher dosage due to increased pressure which caused higher rates of sorption in the wood and more rapid leakage from the jars.

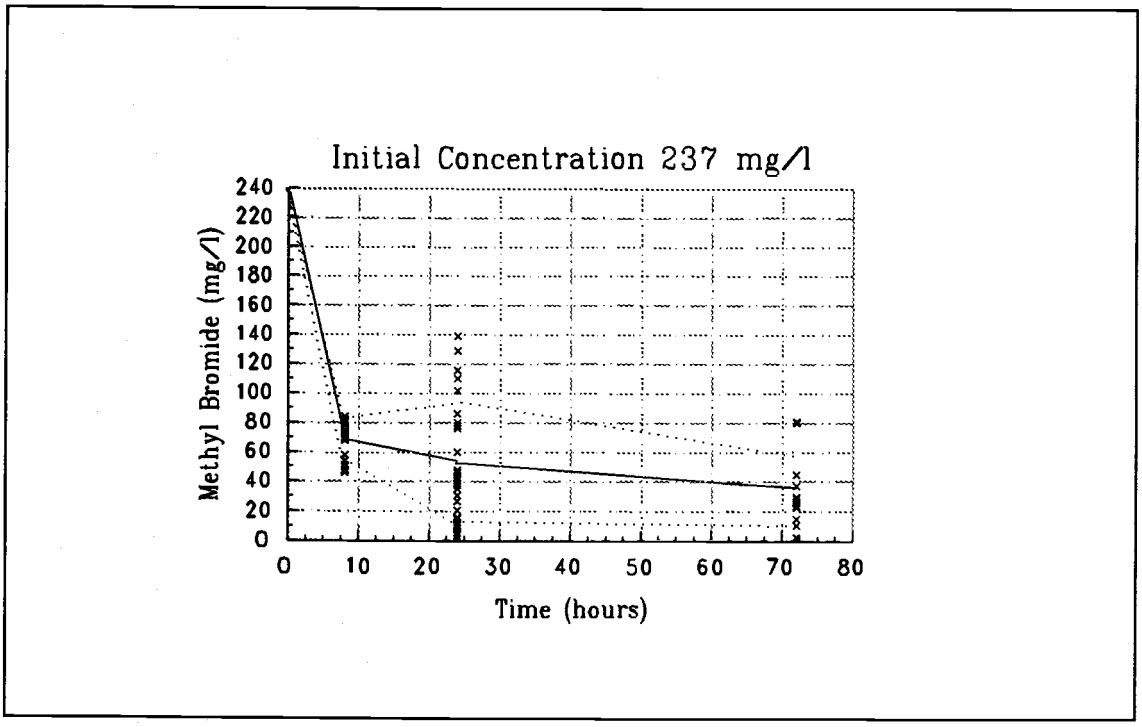

Figure 5. Final concentrations of methyl bromide in jars with initial concentrations of 237 mg/l. Solid line represents mean, dotted lines represent one standard deviation.

After eight hours, the average rates of methyl bromide loss decreased greatly and stabilized after 24 hours. Variability in jars receiving the higher initial concentration was greatest at 24 hours and lowest at eight hours. Part of this observation was due to leakage, as previously described. It is also likely that by eight hours sorption into the wood cubes was proceeding as quickly as the constraints of the materials would allow. By 24 hours, reduced fumigant pressure probably made the previously described effects of leakage and sorption more sensitive to the differences of the wood and containers. By 72 hours these rates had somewhat stabilized as indicated by the reduced variability of the corresponding final concentrations. The methyl bromide sorbed by the wood probably dampened the effects of leakage as it was quickly sorbed and then slowly released over time.

Jars receiving the lower initial fumigant concentrations had similar variability at each fumigation time, but much less precipitous drops in methyl bromide levels from the initial concentration. This probably reflects a lower initial pressure as a result of the presence of one-third as much methyl bromide as that used in the higher concentrations. Diffusion pathways were probably not saturated and the higher variability relative to the lower initial concentration probably reflects wood anatomy and moisture content and seal efficacy more than the higher concentration.

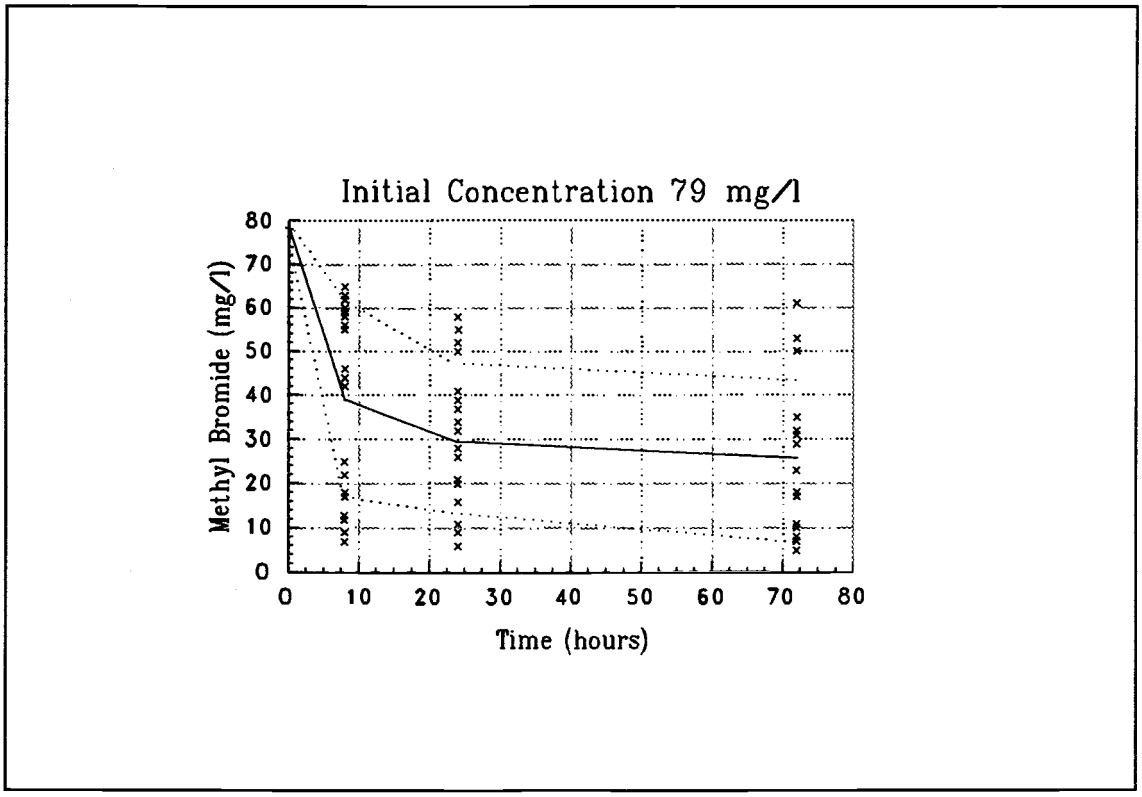

Figure 6. Final methyl bromide concentrations of jars with initial concentrations of 79 mg/l. Solid line represents average concentration; dotted line represents one standard deviation.

As noted, actual cumulative methyl bromide exposures in these tests were much lower than target CxT values due to sorption and leakage. The CxT values reported here were calculated by assuming a linear rate of methyl bromide depletion from initial to final concentration. Although these values were much closer to the actual exposure, they still overestimate cumulative exposure in contrast to steady state laboratory fumigations. The magnitude of this overestimate can be discerned by the slope of the curves for

final concentrations, discussed previously. This overestimate was probably unimportant for eight hour fumigations, but was more important for 24 hour fumigations, and may have been quite large for 72 hour fumigations. The overestimate was also more critical at the higher initial concentration, and for individual replicates with lower than average final concentrations. However, thess overestimates relative to steady state laboratory fumigations may possibly be more accurate for predicting field applications. Schmidt et al. (1982) found that fumigation schedules effective in laboratory chamber fumigations reduced, but did not eliminate the oak wilt fungus in field fumigations of logs. Additional field trials by MacDonald et al. (1985) and Liese and Ruetze (1985) found it necessary to add more gas at 24 hours to restore the fumigant levels to the initial concentrations for complete control.

5. Toxicity of Methyl Bromide to Fungal Species Tested

Due to time and material constraints, usable data over the range of CxT values was limited. Trends in toxicity of methyl bromide to target species were not very evident when considered individually. However, once the data was compiled certain patterns emerged. Therefore, individual species response will be described prior to a summary of overall response.

a. *Armillaria ostoyae*

Results of fumigation at various average concentrations over the three time periods on the mortality of *A. ostoyae* are displayed in Figure 7. They show complete mortality in the three treatment groups with the highest CxT values. The three non-control treatment groups with lower CxT values all have one or more survivor. The steep slope of the line indicates that time is of more relative importance than concentration for successful fumigation. For the small wood pieces being tested, this association is surprising. With larger piece size, longer distance diffusion would require even longer fumigation times for complete mortality. The line shown depicts the CxT values beyond which no survival was recorded. CxT values beyond this line represent minimum values for which complete mortality may be expected. Near each axis the linear relationship would probably break down, and become asymptotic.

The effect of CxT values on percent survival is shown in Figure 8. No survival was detected beyond the solid line indicated on the graph; however, it should be noted that the survival of the controls was quite variable. Survival probably would have been greater had the rhizomorphs and mycelial mats not been scraped off the exterior along with the vermiculite as the cubes were placed in the jars. These fungal structures might have provided an additional barrier to fumigant penetration into the wood.

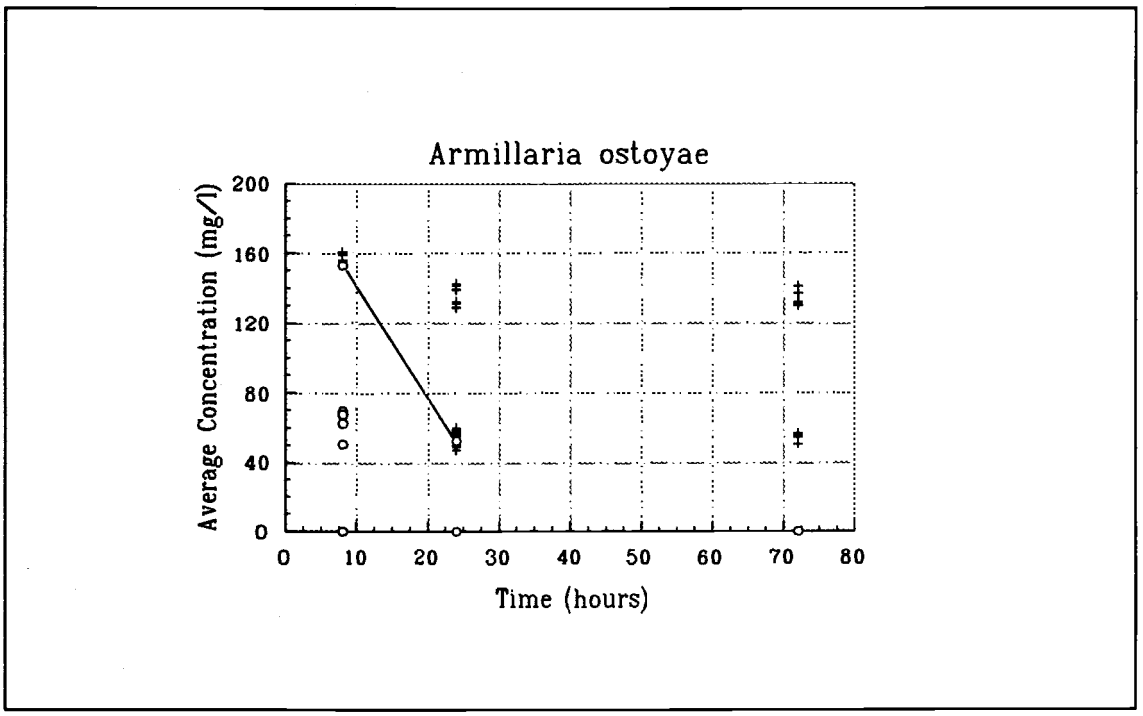

Figure 7. Fumigations tested against *Armillaria ostoyae*; average concentration is plotted for each fumigation time. Crosses represent complete mortality; circles represent survival by one or more subsamples.

A large body of literature has developed concerning the effects of methyl bromide on *Armillaria mellea (sensu lato)*. Munnecke et al. (1978) found that a CxT value of 779 mgh/l controlled 90% of the propagules of this fungus on potato-dextrose agar at 24° C. This compares with the highest CxT value observed in the present study of 1267 mgh/l from which 7% of the subsamples survived. The greater CxT value required by the present study can probably be explained by the lower temperature of fumigation, the higher level of kill reported, the probable poor permeability of saturated larch

wood relative to agar and possible differences in isolate or species susceptibility.

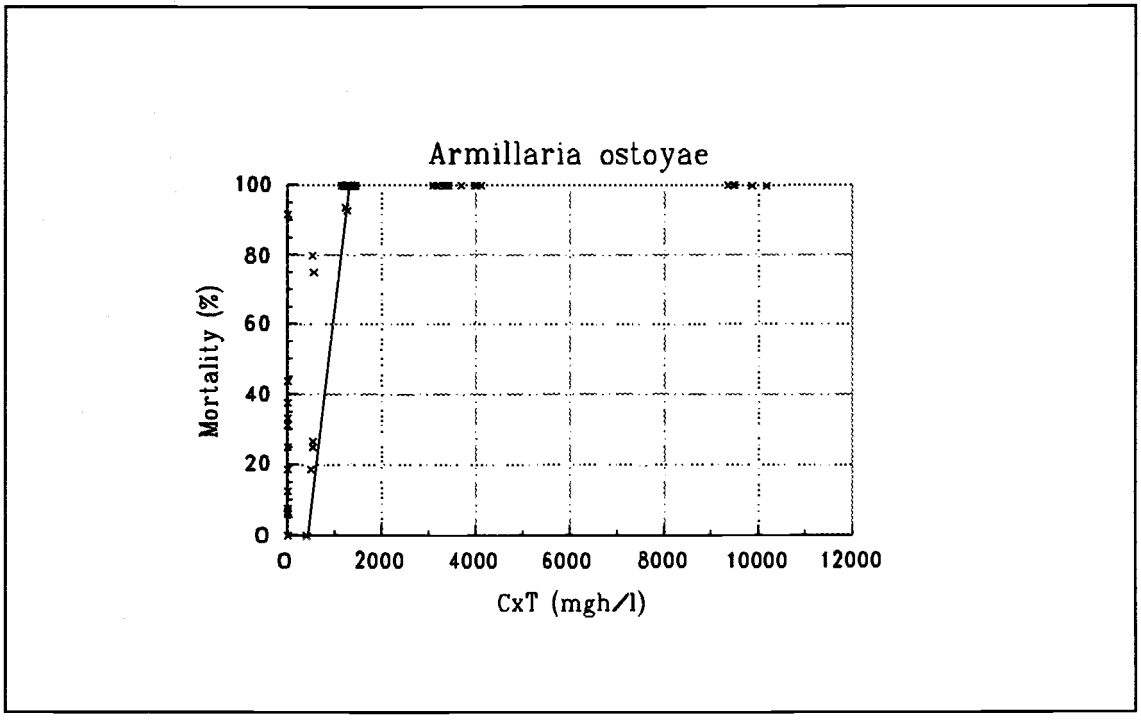

Figure 8. Percent mortality of *Armillaria ostoyae* with increasing CxT values of methyl bromide.

In a related study, Munnecke et al. (1970) fumigated pieces of citrus root inoculated with *A. mellea* at 20° C using a similar fumigation technique. They found that CxT values ranging from 456-461 mgh/l killed 95% of the propagules when the fungi were buried in nonsterile soil. In a later report (1981) they attributed this greater success under more difficult conditions to the antagonistic effects of *Trichoderma viride* Pers. ex Fr. They found the antagonist more tolerant of methyl bromide fumigation than *Armillaria*

and able to attack the pathogen when its defenses were weakened by heat, drought or sublethal fumigation. They theorized that in its weakened state the pathogen was hindered in its ability to form or repair ruptures in its pseudosclerotial walls. Although this form of biocontrol is intriguing and apparently effective in the forest (Filip and Roth, 1977), such methods are insufficiently reliable at this time to use in applications where failure to achieve control involves considerable liability. Also, the antagonist can be found in soil, the air and cavities of trees, but is not common deep in the wood where the pathogen resides (G. Filip, personal communication). Since *Armillaria* can occasionally be found deep in saturated heartwood it is not likely that the fumigation schedules tested in this report will result in CxT values high enough to kill this pathogen under these conditions throughout imported logs.

b. *Heterobasidion annosum*

Results of methyl bromide fumigations at various average concentration and time combinations are shown in Figure 9. Once again the solid line represents the concentration time relationship beyond which no fungi grew from the blocks. In contrast to *Armillaria ostoyae,* only the 72 hour treatment groups had complete mortality. Judging from the decreased slope of the line, concentration had a stronger relative effect on mortality than time with this apparently more resistant species in the small wood cubes tested. However, this could be due to increased growth and penetration of the wood cubes by the fungus, requiring more fumigant over time to penetrate and kill the fungus. Observations at the time

indicated that wood cubes inoculated with *H. annosum* were more obviously decayed than cubes inoculated with the three other species tested.

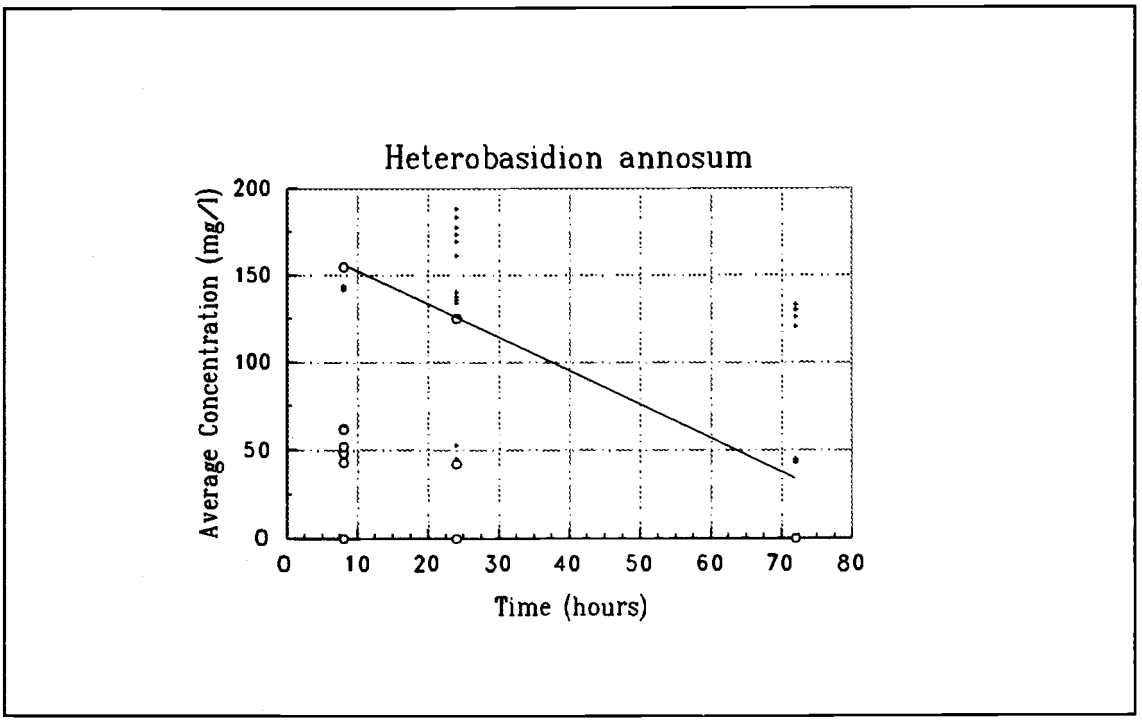

Figure 9. Fumigations tested against *Heterobasidion annosum;* average concentration is plotted for each fumigation time. Crosses represent complete mortality; circles represent survival by one or more subsamples.

The effect of severity of treatment on percentage of fungal mortality is depicted graphically in figure 10. The 6% survival of a single replicate at a CxT value of 3006 mgh/l indicates relative resistance of this species to methyl bromide. Although this measurement can be considered

anomalous, it was the lowest CxT value recorded for this species with the nominal treatments of 5760 mgh/l. Why this fungus survived this treatment is unknown. Houston and Eno (1969) in their soil fumigations of *annosus* root rot noted poor fumigant penetration associated with resinous wood. The larch wood tested in this study often contained very small pitch flecks, and perhaps mycelium was present in such an area. Although this pathogen forms conidia and basidiospores that could be more resistant to fumigation than mycelium, they would most likely be on the surface of the cube and therefore, in a more vulnerable location. Houston and Eno (1969) also noted the rapid colonization of fumigated wood by *T. viride* which could aid the success of field fumigations.

However, according to the limited data available, conventional tent fumigations will not likely deliver accumulated dosages necessary to destroy *H. annosum* in the wet, resinous, partially decayed heartwood of refractory species such as Dahurian larch. Therefore, some other mitigation measure will probably be necessary to preclude the entry of this fungus with imported logs.

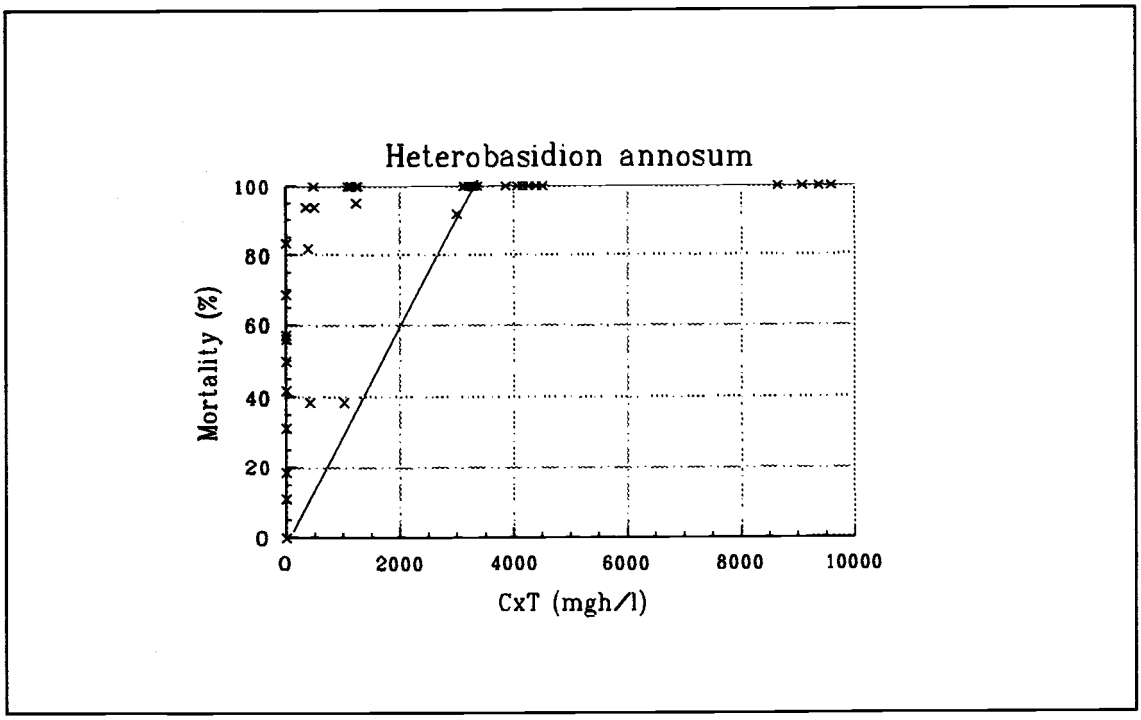

Figure 10. Percent mortality of *Heterobasidion annosum* with increasing CxT values of methyl bromide.

c. *Lachnellula wilkommii*

Data for the relative efficacy of average concentration and time of fumigation against this species are shown in Figure 11. As with *A. ostoyae*, the three highest treatment groups completely eliminated this fungus from the test cubes. Also in common with *A. ostoyae*, the steep slope of the line indicates greater relative importance of increased time of exposure in obtaining complete mortality. The larch canker fungus appears to be one of the more susceptible species from the limited data recorded. However, the survival of this

pathogen at the lower initial concentration for 24 hours indicates that the T404 fumigation schedule, used for destroying wood boring insects in wood (USDA, APHIS, 1991) will probably be ineffective against this fungus.

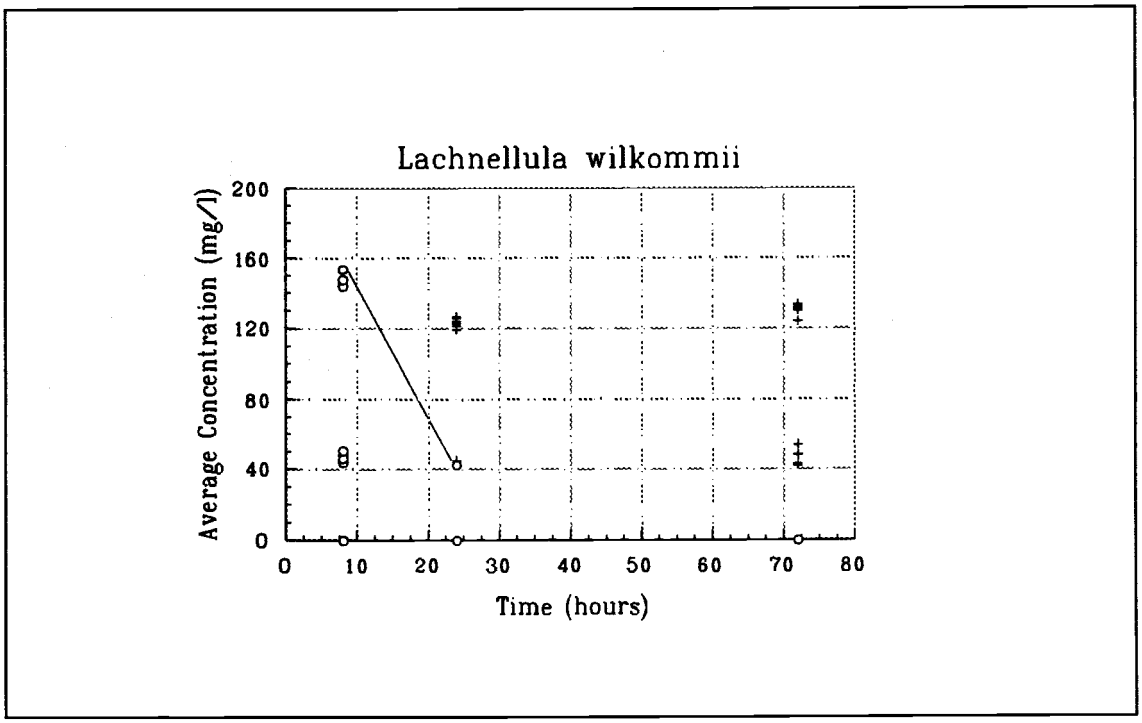

Figure 11. Fumigations tested against *Lachnellula wilkommii*; average concentration is plotted for each fumigation time. Crosses represent complete mortality; circles represent survival by one or more subsamples.

Figure 12 shows percent mortality as a function of CxT values for this species. Its response was quite different from the other species; no survival was recorded beyond 1230

mgh/l, although survival was quite high at levels less than 100 mgh/l below that.

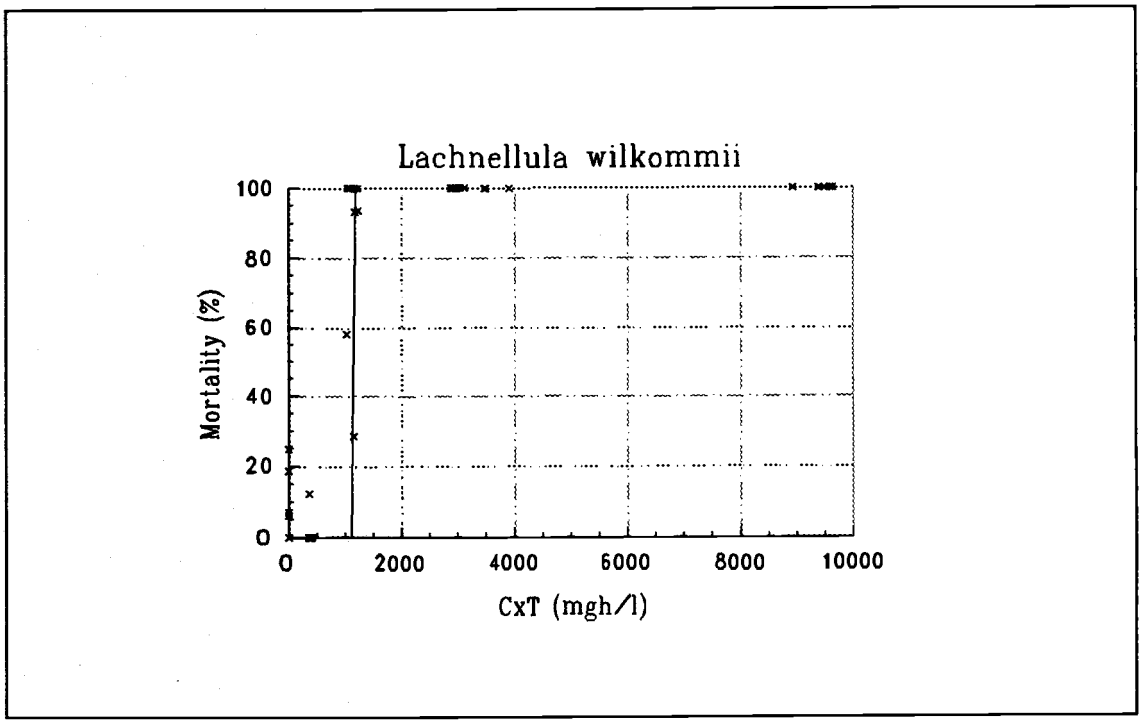

Figure 12. Percent mortality of *Lachnellula wilkommii* with increasing CxT values of methyl bromide.

The relatively narrow range for lethality could be due to a number of factors. Possibly, more samples would have revealed survival at higher levels. A threshold exposure may have been reached, beyond which the fungus could not survive. Alternatively, the fungus may not have colonized the wood deeply. Hiley (1919) noted very poor penetration of the fungus in wet sapwood with insufficient aeration. He also noted that the fungus flourished once it penetrated to the drier heartwood. The tree responded to this infection by

producing gums and resins, both of which would probably impede fumigant penetration of imported logs. The cubes tested were saturated heartwood and were probably not deeply penetrated by the fungal mycelium.

L. wilkommii produces microconidia and ascospores for dispersion and these propagules may offer some resistance to environmental stress. However, these spores will likely form on the surface of the wood and thus, be exposed to more fumigant than the mycelium deep in the wood. Methyl bromide fumigation will probably penetrate to the depth necessary to kill the superficial infections in the sapwood, but not those deeply imbedded in resin and gum saturated heartwood.

d. *Leptographium wageneri*

Mortality relative to different average concentrations and exposure times for this pathogen is summarized in Figure 13. Once again, the highest target CxT value group produced complete mortality. Also readily apparent, was the incomplete mortality after a 72 hour fumigation. This was the only 72 hour fumigation in which survival occurred. However, fumigation with the same initial concentration was completely successful at 24 hours.

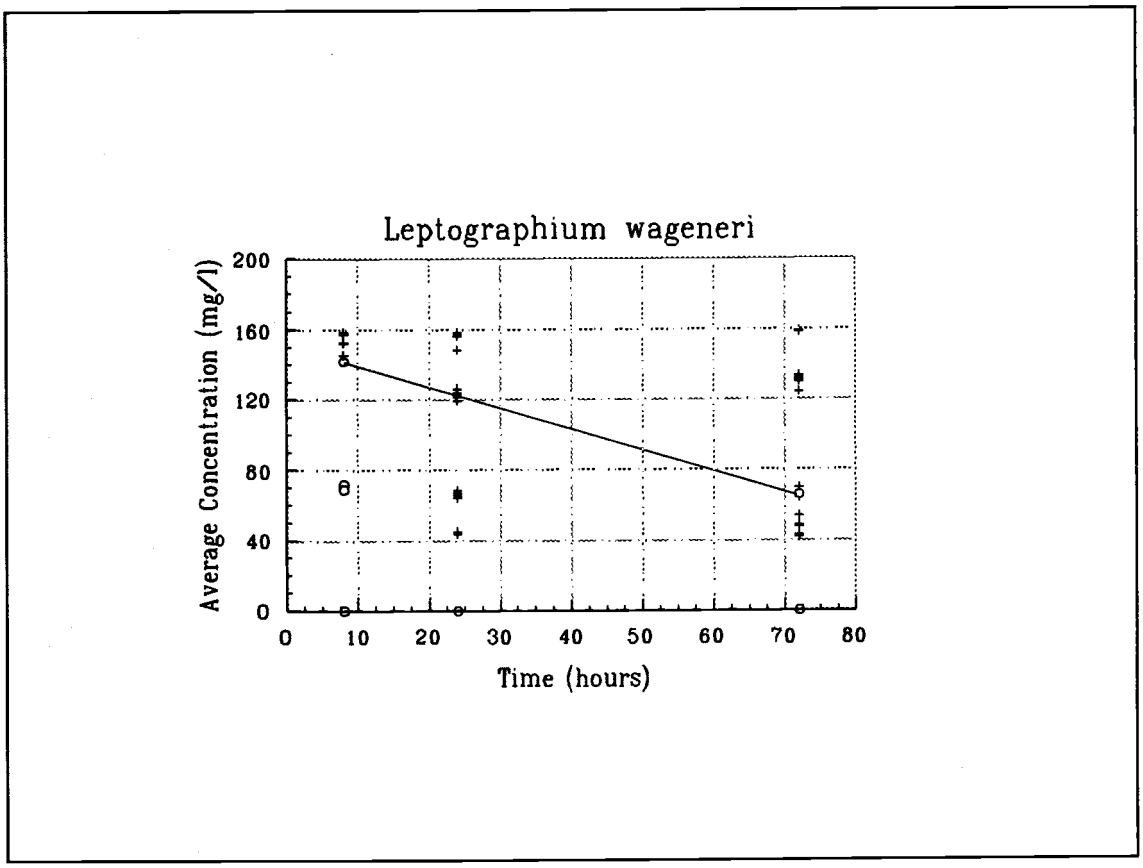

Figure 13. Fumigations tested against *Leptographium wageneri;* average concentration is plotted for each fumigation time. Crosses represent complete mortality; circles represent survival by one or more subsamples.

Figure 14 shows the incomplete mortality of 87.5% at a CxT value of 4748 mgh/l. The survivors of this replicate were two quarter sections from different cubes. This occurrence would lead one to believe that survival was not due to a chance event affecting random cubes, but rather to a factor that affected many of the cubes of this treatment. Coincidentally, the cubes for this treatment came from the

inoculation bag which had the highest moisture content sample of the refined tests (148%). The target fungi could have grown deeply into the saturated cubes and been more protected than in blocks with lower moisture contents. Most surprisingly, this replicate had the second lowest leakage of any replicate in the 79 mg/l for 72 hour treatment group; 79% of the fumigant was in the wood or the air of the jar at the end of treatment time.

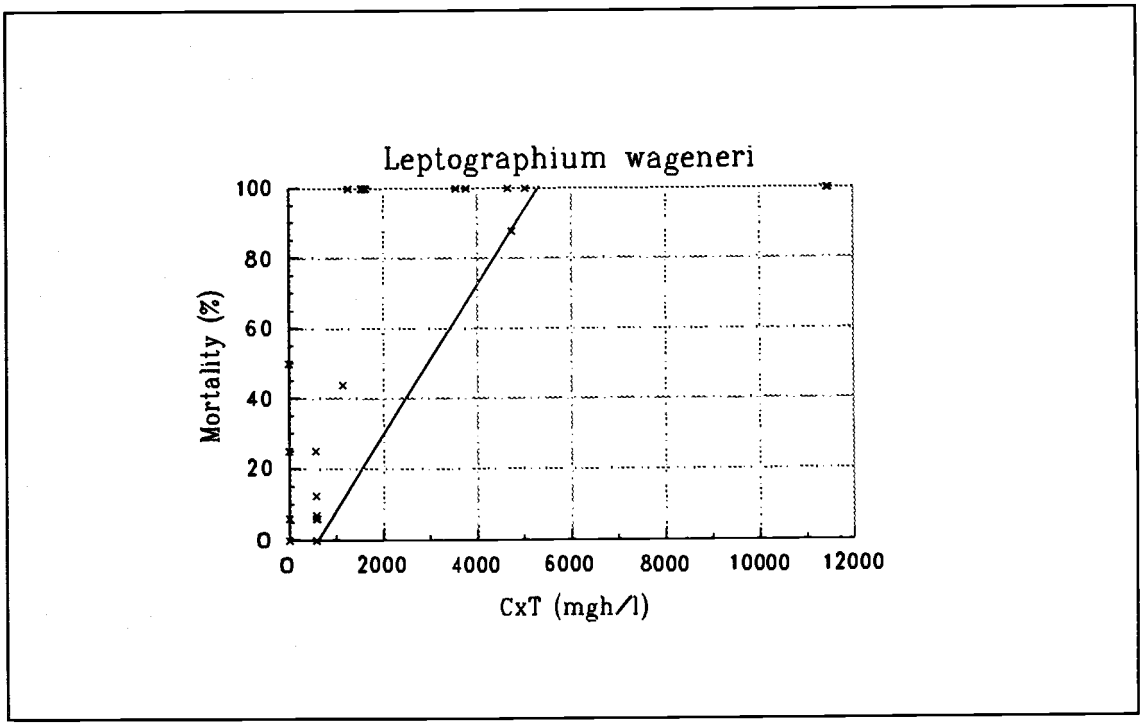

Figure 14. Percent mortality of *Leptographium wageneri* with increasing CxT values of methyl bromide.

In Figure 14, a considerable distance was evident between the outlying point referred to and the next lower one. The next lower point has 44% mortality at a CxT value

of 1138 mgh/l which is less than one-fourth that of the outlier. This large gap between these values was probably an unfortunate consequence of a small sample size made even smaller due to contamination of both bags of wood cubes for the first 72 hour series of fumigations. Only one bag was available for replacements, hence only three replicates were available for each initial concentration.

Paradoxically, since larch sapwood is usually less than 2.5 cm thick (Panshin and deZeeuw, 1980), CxT values high enough to kill this species or similar sapwood staining fungi, probably will be reached using the T312 fumigation schedule which is effective against the oak wilt fungus. However, it should be stressed that a viable fumigation schedule for Dahurian larch logs must be effective against a wide range of pests throughout a log. Schmidt et al. (1982) found decay fungi present in sapwood of oak logs that had been successfully fumigated in chambers for 72 hours with 240 mg/l of methyl bromide. Other dangerous pathogens in larch sapwood may resist similar fumigation schedules.

e. All Species Combined

Figure 15 shows the relationship between concentration and time for all the fumigations for which usable data was recorded. A fairly good linear relationship exists between the most resistant survivors over the ranges of both these factors tested. Values beyond this line represent target CxT values to be achieved throughout logs in field fumigation trials for a realistic chance of pest elimination.

Whether these targets can be achieved with conventional tent fumigations is another matter. Initial concentrations

greater than 240 mg/l are not recommended by USDA, APHIS due to the difficulty of safely handling these concentrations in field fumigations under plastic tarpaulins (Liese and Ruetze, 1985). Since fumigations requiring a three day exposure can easily require over a week to set up, fumigate and vent, a longer time period is not considered to be practical either (Schmidt et al., 1982).

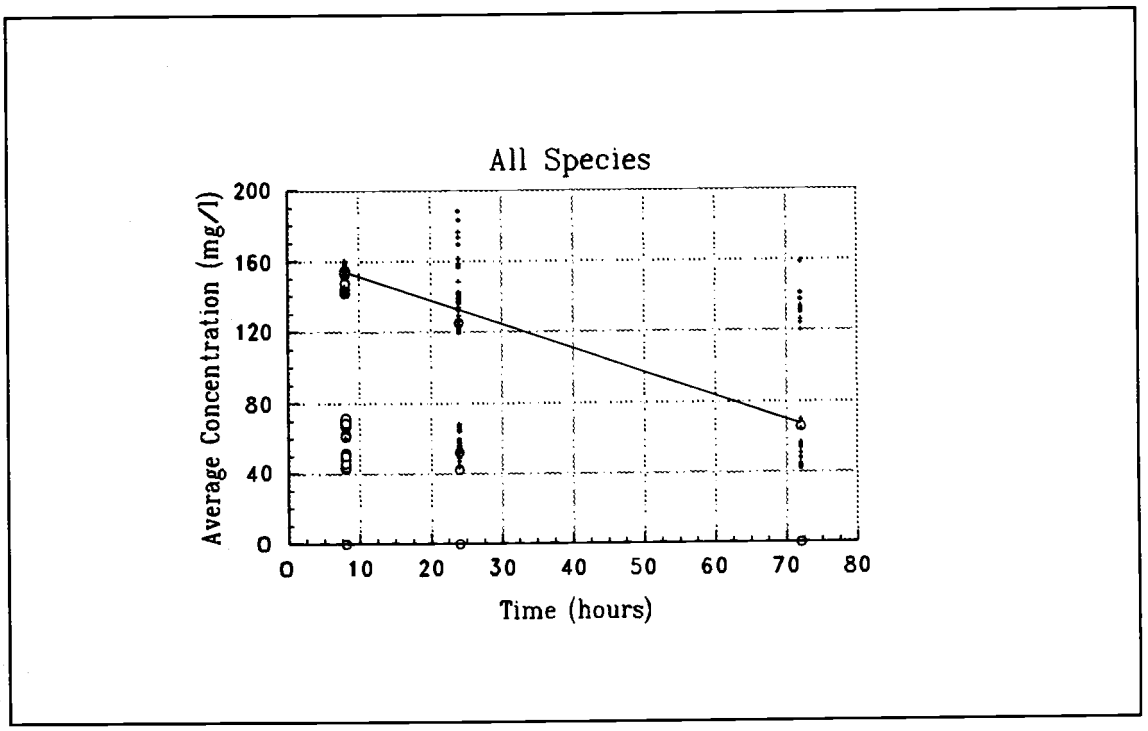

Figure 15. Fumigations tested against all species; average concentration is plotted for each fumigation time. Crosses represent complete mortality; circles represent survival by one or more subsamples.

Figure 16 shows the mortality of all fumigations with respect to CxT values. The relative success of most

fumigations with CxT values over 2000 mgh/l stands in contrast to the two survivors above that level. However, these observations were consistent with survival of selected fungi in previous laboratory fumigation reports.

The low level of the survival of some of the controls indicated that inoculation was not completely consistent. Cubes with superficial infestations were likely quickly killed, while the most resistant survivors probably had hyphae and other fungal survivor structures that deeply penetrated into wet, resinous cubes.

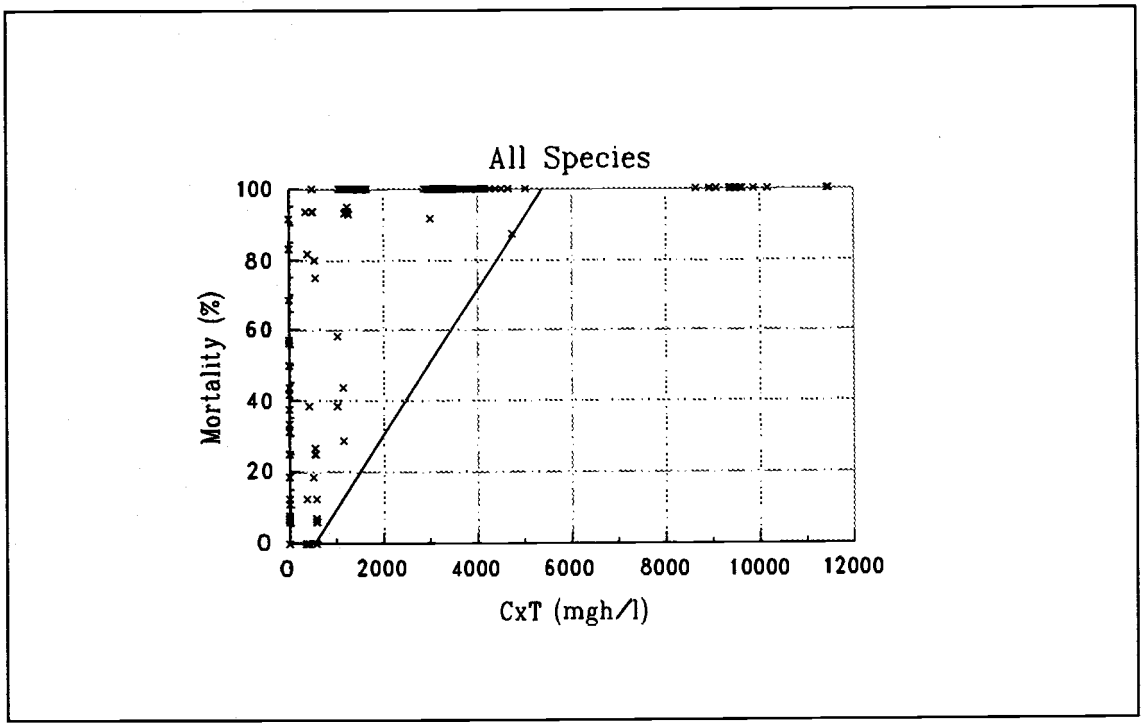

Figure 16. Percent mortality for all species with increasing CxT values of methyl bromide.

VI CONCLUSIONS

Several options are available to improve the chances of successful of fumigation of imported logs over that of conventional tent fumigation techniques. The easiest variable to manipulate is sample dimensions, particularly length. Shorter logs allow relatively rapid longitudinal fumigant diffusion through a greater proportion of a log than longer logs. Squaring logs into cants would remove bark and most of the relatively wet sapwood while reducing piece size. Both of these techniques will also hasten the drying process which will in turn, improve penetration.

However, these techniques are not without their drawbacks. Reducing sample dimensions will require more labor and limit the range of products that can be manufactured from a given log. Proper air drying takes a considerable amount of time, particularly with large pieces of timber. Large pieces are also more likely to sustain drying defects than smaller pieces.

Fumigation could also be made more effective by increasing the temperature of the logs. Although the fumigations in this study were conducted at 18° C, lower CxT values may well achieve better results at slightly higher temperatures. Sorption would be reduced (Monro, 1969), and increased respiration of target organisms may make them more vulnerable to fumigation. However, providing heat would probably be too expensive in most cases.

Performing fumigations in a vault or ship hold would probably provide for a considerable increase in fumigant retention over tent fumigations (Cross, 1991). However, considerable expense may be incurred constructing a vault or

fitting out a ship for such a purpose (USDA, APHIS, 1991). Most ships which transport wood products are not used exclusively for this purpose, further reducing the incentive to construct special facilities.

It may take considerably more research to find an effective, economically viable fumigation technique for imported Russian timber. Munro (1969) makes the case that successful fumigation of a given commodity at various conditions cannot be predicted from known laws and generalizations and that empirical data are a more reliable base for treatment recommendations. Field trials under various conditions should be proven effective before methyl bromide fumigation of Dahurian larch logs can operationally proceed.

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APPENDICES

Appendix A

Pest Species Introduced on Wood Products

ISOPTERA

Rhinotermitidae

Coptotermes formosanus (Shiraki), the Formosan subterranean termite was introduced into Hawaii about 1900 where it has since caused severe damage. Walls in new buildings have been hollowed out in as little as three months. Damage in Honolulu alone was estimated at between $2 and $3 million annually (Beal, 1967). In 1965, this species was discovered in a Houston, Texas shipyard by a pest control operator. Subsequent infestations were found in New Orleans and Lake Charles, Louisiana, Galveston, Texas and Charleston, South Carolina (Anonymous, 1967). In 1980 the pest was found in southern Florida (Thompson, 1985). Original infestations were presumably from equipment and cargo from overseas. The infestations in New Orleans were located in an army transportation center which was largely dismantled and moved to other areas prior to detection (Gentry, 1966). According to Beal, the potential range of the termite is along both coasts as far north as Boston, Massachusetts and (from Kofoid, 1934) Tacoma, Washington. Gentry estimated the potential damage at $62 million per year. Morrell (personal communication) states that damage from subterranean termites in the U.S. is approximately $ 1 billion per year.

Kalotermitidae

Cryptotermes brevis (Walker), the house drywood termite was introduced in about 1920 to southern Florida from the nearby islands. Although unknown, the likely vector was infested wood products or debris (Minnick et al., 1972). This species has also been introduced in Hawaii, Louisiana and various parts of Africa (Wallenmaier, 1989). The species has been intercepted in seasoned timber in Queensland, Australia (Wylie and Yule, 1977) and is now established there (Wallenmaier, 1989). According to Hickin (1971) it is a serious pest in buildings in southern Florida where, along with the native drywood termites economic losses from this family of insects are perhaps the heaviest in the world. Since this species commonly infests furniture, it is easily moved in commerce and small infestations have been found in other states (Wallenmaier, 1989).

COLEOPTERA

Oedemeridae

Nacerdes melanura (L.), the wharf borer is, as it name suggests very destructive to wharves, pilings, boardwalks and damp basement timbers. It was introduced from Europe and is now widely distributed in North America on both coasts and around the Great Lakes (Baker, 1972).

Anobiidae

Anobium punctatum (DeG.), the common furniture beetle has been established in the northeastern United States and southeastern Canada with scattered locations along the Southeastern, Gulf and West coasts. Damage is most serious in old wood such as antique furniture (Baker, 1972).

Xestobium rufovillosum (DeG.), the deathwatch beetle is a European species that is now widely distributed in the northeastern United States. It is a long-lived insect that is very destructive to both heartwood and sapwood of old timbers in buildings. It is considered one of the most serious pests of wood in buildings (USDA, 1954).

Cerambycidae

Monochamus alternatus Hope, the Japanese sawyer is a native insect that is the vector for the introduced pine wood nematode (*Bursaphalenchus xylophilus* [Steiner & Buher]) in Japan. It is a major pest complex infesting 25% of that nation's 2.6 million ha of pine forests (Mamiya, 1983). The heaviest annual loss was in 1979 when 2.4 million cubic meters of timber was destroyed (Mamiya, 1987). *Pinus densiflora* Sie. & Zucc. and *Pinus thunbergii* Parl. are severely affected, particularly on hot, dry sites. It was first described in 1934 by Steiner and Buher in east Texas on longleaf pine (*Pinus palustris* Miller) timber, and has the distinction of being the first wood inhabiting nematode to be discovered. It was introduced into Japan about 1905 near Nagasaki, but for over 60 years its effects were attributed to its insect vector. In 1969 it was first reported on dying pine trees in Japan by Tokushige and Kiyohara; in 1971 the same authors suggested that it was the causal agent of pine wilt disease. In 1973 the native pines of Okinawa Island (*Pinus luchuensis* Mayr) were found to be attacked by the same pest complex; spread was accomplished by means of infested logs (Mamiya, 1983). In 1982, the pine wood nematode was found in Nanjing, China on dead and dying Japanese black pine. Nearby Masson pine (*Pinus massoniana* Laub.) was unaffected. It was found in 1985 in Taiwan on an

exotic plantation of Okinawa pine that was suffering 50% mortality (Mamiya, 1987).

In North America it attacks mainly exotic hard pine species, the native species being resistant. It is a major concern for North American forest products exporters however. Currently the European Economic Community requires pasteurization of unprocessed softwood products from North America because of concern over accidental introduction. Finland was the first country to enact such requirements after finding the pathogen in wood chips from North America (Rautapaa, 1986).

Hylotrupes bajulus (L.), the old house borer has been established in the eastern United States, Africa, Australia, southern South America and South Africa. It has been present in the United States for over 130 years where it damages sound softwood of both old and new houses. It prefers sapwood and can cause roofs or other structural members composed primarily of sapwood to collapse. Buildings in the United States have been condemned due to damage from this insect (USDA, 1961).

Arhopalus tristis (Mulsant), the burnt pine longhorn borer was introduced into New Zealand from Europe (USDA, Forest Service, 1992). It is found in dead, primarily burned trees and logs. It was first detected in 1963 in New Zealand, and by the early 1970s was reported to be widespread. It is of economic concern in New Zealand primarily because it has been found on packets of timber on wharves and in mills where it damages timber, and could cause rejection of export timber (Forest Research Institute, 1973).

Phoracantha semipunctata (Fabricus) and *Phoracantha recurva* (Newman), are eucalypt borers that probably entered South Africa on freshly cut railway sleepers imported from

Australia during the Boer War. They were first recorded near Wolsely in 1906 and are now found throughout South Africa wherever eucalypts occur. They are usually found on dead trees and felled logs, but can kill stressed trees primarily during drought (Cillie and Tribe, 1991). *P. semipunctata* has since been introduced to California probably via infested dunnage. It was first discovered in 1984 in Orange County. Since that time it has spread to all Southern California counties and three Bay Area counties. In California, eucalyptus borers can kill drought stressed trees much more readily than in their native Australian habitat. They have necessitated costly tree removals in urban areas and have made some plans to manage eucalypts on short rotations for pulp or biomass uneconomical (USDA, Forest Service, 1991).

Scolytidae

Blastophagus minor (Hartig) is listed by Marchant and Borden (1976) as being introduced from the Palaearctic region to China. The mode of establishment is unknown, but given its occurrence on pine, spruce and fir, introduction via wood products is not unlikely.

Blastophagus piniperda (L.) is listed by Marchant and Borden (1976) as being introduced from the Palaearctic region to Japan. The mode of establishment is unknown, but given its occurrence on pine, spruce and larch in both native and introduced ranges introduction via timber trade is not unlikely. The insect was first discovered in the U.S. near Cleveland in 1992. It probably arrived from Europe on infested packing crates (Bridges, 1993).

Since that time *B. piniperda* has been found in 44 counties in six states. In 1993 APHIS issued an interim rule to regulate the shipment of pine nursery material, Christmas

trees and logs from infested areas. This led to a great deal of disruption in the local trade of these areas (Nielsen, 1993). The ultimate effects on the forests and landscapes of North America is unknown.

Coccotrypes indices (Eggers) is listed by Marchant and Borden (1976) as being introduced from the East Indies to the West Indies on fruit and the bark of Mahogany. *Swietenia* spp. which only occurs in the New World is listed as a host as well as "many other species". They list it as probably established in Florida in 1975.

Dendroctonus micans (Kug.) is a pest of spruce that has greatly increased its range over the past 100 years. It was probably brought into France from northeastern Europe on unbarked wood in 1952 (Marchant and Borden, 1976), and Soviet Georgia by the same means in 1957. In 1982, it was discovered to be well established on Sitka spruce in Wales. Surveys of dead trees dated the original infestation to 1973 (Fielding et al., 1991). In spite of emergency sanitation felling and restrictions on movement of infested timber and biological control attempts, the beetle has spread throughout most of Wales and the English Midland District (King and Fielding, 1989).

Crypturgus pusillus (Gyllenhall) is an Old World native of spruce that was probably brought to the northeastern United States or eastern Canada in the colonial period. It is a secondary enemy of spruce that breeds in the bole and larger branches of the host (Wood, 1977).

Gnathotrichus materiarius (Fitch) is an ambrosia beetle that is secondary enemy of Pinaceae. It is a native to eastern North America that was recorded in France shortly before 1936 (Wood, 1977). It has since spread to the Netherlands and Germany (Marchant and Borden, 1976).

Hylastes angustatus (Herbst) is a pest of pines that is native to Europe and Japan. It has been established in South Africa and Swaziland, possibly in logs or dunnage (Marchant and Borden, 1976). In South Africa, females feed on the bark of pine seedlings during their maturation feeding phase. Over 50% of newly planted pine seedlings may be killed by the beetle girdling the cambium. Adults overwinter in stumps or butts of logs and may be transported to other sites after harvest (Tribe, 1991).

Hylastes ater (Paykull) is a pest of a wide range of conifers. It is native to the Palaearctic, and has been established in Australia and New Zealand (Marchant and Borden, 1976) and Chile (Cisela, 1988). In these countries, the beetles frequently damage seedling stands. In Chile, up to 70% of the seedlings in naturally restocked stands have been destroyed. This species has also been implicated in mortality of regeneration in New Zealand, but export of infested logs is the primary cause for concern. According to New Zealand law, infested logs must be fumigated before shipping. It is estimated that fumigating *H. ater* infested logs costs $250,000 per year in the Bay of Plenty alone (Zondag, 1979). In Australia, the worst recorded attack was in a Victoria plantation where all seedlings in a 3.2 ha plantation were killed (Neumann, 1987).

Hylastes attenuatus (Erichson) is considered by Marchant and Borden (1976) to be an introduced species in Japan. Logs have been implicated as a possible means of long distance spread. This pine pest is native to Europe.

Hylastes linearis (Erichson) is listed by Marchant and Borden (1976) as being a native of Europe established in South Africa. Its hosts are pines in both its native and introduced ranges, but the mode of introduction is unknown.

Hylastes opacus (Erichson) is listed by Marchant and Borden (1976) as being a native of the Palaearctic established in South Africa. Its hosts are also pines in both native and introduced ranges, and its mode of introduction is also unknown.

Hylastes pinastri (Eggers) is listed by Marchant and Borden (1976) as being a native of Europe; it too is established in South Africa. Its hosts are also pines in both native and introduced ranges, and its mode of introduction is also unknown.

Hylurgus ligniperda (Fabr.) a native to Europe and the Mediterranean is now established in Japan, South America, South Africa, New Zealand (1974), Australia (1942), Sri Lanka and Swaziland. It is a pest of the pines and was probably introduced into most of the afore-mentioned countries via dunnage and logs (Marchant and Borden, 1976) or packing crates (Cisela, 1988). In South Africa, it is considered a minor pest; damage is usually restricted to blue stain fungi in brood galleries (Tribe, 1991). In Chile, it causes seedling mortality in the same manner as *H. ater,* although damage can also occur in trees up to ten years of age that have been weakened by other damaging agents (Cisela, 1988). In Australia trees up to 14 years old have been killed (Neumann, 1987). In New Zealand, damage is very similar to that of *H. ater,* and fumigation of export logs is also required (Zondag, 1979).

Ips calligraphus (Germer) is a pest of pines which has been established in the Philippines and California on logs from the eastern United States (Marchant and Borden, 1976). Although it is usually a secondary pest of pines in the United States, in the Philippines it is a primary invader as large populations can develop on weakened or dead trees.

This beetle has been responsible for substantial mortality of Benguet (*Pinus merkusii* Jung. & de Vrise) and other pines in north and central Luzon since 1958 (Yamaguchi, 1979).

Ips cembrae (Heer) is a native of the Palaearctic which was introduced into Scotland on German timber imported from 1946 to 1948. In 1955, it was found in 29 plantations where it had girdled twigs and killed many trees outright. The most severe damage was in wind damaged plantations, where standing trees as old as 45 had been killed, (Crooke and Bevan, 1957). It is now found attacking weakened larch trees throughout Scotland. An associated stain fungus (*Ceratocystis laricicola* Redfern & Mintner) has been implicated in mortality of larch plantations. It is believed that this pathogen is vectored by *I. cembrae* and was introduced to the U.K. at the same time (Redfern et al., 1987).

Ips grandicollis (Eichhoff) was imported to South Australia from the eastern United States in 1943 on pine timber with the bark on. In 1952, it had been introduced into Western Australia by the same means (Morgan, 1967). In 1982 it was reported in Victoria where mass infestations have occurred in sapling-sized stands on marginal sites, particularly near previously logged areas. Mature trees that have been wind or lightening damaged have also been killed (Neumann and Morey, 1984). In 1983 it was recorded in New South Wales near the heart of the radiata and slash pine growing region (Neumann, 1987). Blue staining and associated degrade of logged or beetle killed timber is also a common problem since the beetle has associated stain fungi (*Ophiostoma ips* [Rumb.] Nannf.) which was also introduced from the United States (Stone and Simpson, 1987).

Ips interstitialis (Eichhoff) is a pest of pines native to Central America and the West Indies. According to Marchant and Borden, (1976) it was introduced into the Philippines by the U.S. military during World War II, and is now well established.

Ips sexdentatus (Boerner) is a species with a wide Palaearctic distribution that according to Marchant and Borden (1976) was introduced into Britain between 1946 and 1948 on timber from Germany and France. It is a pest of pines that is now established there.

Leperisinus varius (Fabricus) is a species with a wide Palaearctic distribution that may be established in Brazil. The mode of introduction is unknown. It affects many northern hemisphere hardwood species (Marchant and Borden, 1976).

Orthotomicus caelatus (Eichhoff) is a native of eastern North America which may be established in Australia. This species is a pest of larch, spruce and pine which may have been introduced via logs (Marchant and Borden, 1976).

Orthotomicus erosus (Woll.) is a native of the Mediterranean Region which has become established in Britain (Marchant and Borden, 1976), Chile (Cisela, 1988) and South Africa (Tribe, 1991). This pine pest is not considered aggressive in Chile. However, in South Africa it is considered a primary pest of trees stressed by adverse climatic conditions. The mode of introduction into exotic environments is unknown.

Phloesinus cupressi (Hopkins) is a secondary pest of Cupressaceae and Taxodiaceae which was introduced into Panama, Australia (1947), and New Zealand (1943) from western North America by unknown means (Marchant and Borden, 1976).

In Australia it causes occasional mortality of *Cupressus* (Neumann, 1987).

Phthorophloeus spinulosus (Rey) is a pest of fir and spruce that was introduced from northern or eastern Europe to France by unknown means. It was noted to be established in 1918 (Marchant and Borden, 1976).

Pityokteines curvidens (Germar) is a pest of fir, larch, spruce and pine that was introduced from Europe or the Orient to South Africa, where it affects pine. It was vectored by unknown means (Marchant and Borden, 1976).

Polygraphus rufipennis (Kirby) is a pest of the Pinaceae that was probably established in South Africa from pine log shipments from Canada (Marchant and Borden, 1976).

Scolytus multistriatus (Marsham) is native to Europe and Siberia; it has been introduced into North America, Australia (Marchant and Borden, 1976) and New Zealand (Forest Research Institute, 1990a). Establishment into North America in 1933 via elm veneer logs from Europe brought its associate, the Dutch elm disease fungus (*Ophiostoma ulmi* Buism.). This fungus was also soon vectored by the less aggressive native elm beetle *Hylurgopinus rufipes* (Eighh.) in addition to *S. multistriatus*. This establishment of an exotic disease and its insect vector led to mass mortality of forest and urban elms. In the United States alone $11 million dollars was spent in the 1930's in a futile attempt to eradicate the disease. By 1977, 60% of the estimated 77 million elms planted in urban areas had been killed (USDA, Forest Service, 1991). The cost of elm disease in terms of tree removal, losses in real estate value and disease control approaches $100 million annually (Stipes and Campana, 1981). More aggressive strains of the disease (the North American aggressive) were reintroduced into Britain on rock elm logs

from Canada in the mid 1960's (Braiser and Gibbs, 1973). This initiated a massive epiphytotic that destroyed 70% of the 22 million elms in Britain (Stipes and Campana, 1981). The aggressive strain has since been introduced onto the European continent from England, and another strain (the European aggressive) has been introduced from eastern Europe. Log movement is the primary means of long distance spread, while association with *S. multistriatus* along with other native *Scolytus* beetles represents the primary means of short distance spread.

The North American strain of Dutch elm disease was recently discovered in New Zealand along with its primary vector *S. multistriatus*. A large eradication program was immediately initiated which appears to have been successful (Forest Research Institute, 1990a). *Scolytus multistriatus* was discovered in Victoria, Australia in 1974 despite stringent quarantine measures, and it is now widely distributed in that state. Losses are light, however because the associated fungal pathogen is not yet present (Neumann and Minko, 1985).

Xyleborinus saxeseni (Ratzeburg) is, according to Wood (1977), native to Europe but, has become established over much of North America, Japan and New Zealand on logs, lumber and dunnage (Borden and Marchant, 1976).

Xyleborus affinis (Eichhoff) was originally native to tropical America but, it is now found in most tropical and some temperate countries. It was introduced into Australia by unknown means in 1929 (Marchant and Borden, 1976). It is a significant pest of logs (Wood, 1977) and very destructive to sweetgum trees (*Liquidambar stryacaflua* L.) in the Gulf Coast States (Baker, 1972).

Xyleborus badius is native to Korea and Japan. It has been introduced into Cuba, South Africa and west Equatorial Africa. This hardwood pest was introduced to South Africa on logs (Marchant and Borden, 1976).

Xyleborus compressus (Lea) is listed by Marchant and Borden (1976) as being native to Australia, and was introduced to New Zealand in 1974 or 1975 in green timber or logs. Its hosts are listed as radiata pine and Douglas-fir, neither of which is native to Australia.

Xyleborus crassiusculus (Motschulsky) is a pest of hardwood trees which is listed by Marchant and Borden (1976) as being native to east Africa, the East Indies, Japan and Korea. It was introduced to Hawaii in 1956 and South Carolina in 1974 by unknown means.

Xyleborus ferrugineus (F.) is listed by Wood (1976) as probably being native to tropical America, although it is now present over much of eastern North America, Hawaii, Micronesia, Australia and Africa. It is a relatively aggressive species.

Xyleborus rubricollis (Eichhoff) is native to Southeast Asia from Malaysia to Japan and Korea and was discovered in Maryland in 1942. It has both hardwood and conifer hosts, and was introduced by unknown means (Marchant and Borden, 1976).

Xyleborus torquatus (Eichhoff) is native to tropical America which has become established in Australia and Japan by unknown means. Its has many deciduous hosts (Marchant and Borden, 1976).

Xyleborus truncatus (Erichson) is native to eucalypts and podocarps in eastern Australia. It has been established in New Zealand by unknown means (Marchant and Borden, 1976).

Xyleborus validus (Eichhoff) was named from Japan and has been reported from Taiwan and China. It became established in New York in 1975, and is often intercepted in packing crates from Japan (Wood, 1977).

Xyleborus volvulus (Fabricus) has a native range from the southeastern United States to Argentina, and has spread to Hawaii, the East Indies, Australia and Africa. Its habits are similar to *X. affinis* and *X. ferrugineus* (Wood, 1977).

Xyleborus xylographus (Say) is a native to eastern North America which was recently introduced to California. It usually breeds on oaks (Wood, 1977).

Xylechinus pilosus (Ratzeburg) is native to Siberia and eastern Europe. It was found to be established in France in 1918 where it was probably introduced on imported logs (Marchant and Borden, 1976). Its hosts in both native and exotic environments are pine, spruce and fir.

Xylosandrus compactus (Eichhoff) is probably of Asian origin. It now occurs in Africa, Florida (1941), Cuba (1958), Hawaii (1961) and Mississippi (1968). The mode of establishment is unknown (Wood, 1977). Wherever it is found it attacks a wide variety of host trees; it reportedly kills healthy established trees of many species in Hawaii (Nelson and Davis, 1972).

Xylosandrus germanus (Blanf.) is believed to be native to Japan. It was introduced into the United States on woody grape cuttings about 1930 and into Germany on oak timber about 1910. It has a wide variety of coniferous and deciduous hosts (Marchant and Borden, 1976). In the United States it is currently a problem on black walnut plantations in the central states. Although healthy trees are seldom killed, growth losses of young trees are potentially serious (Weber, 1981). Kessler (1974) reported a strong association

of this ambrosia beetle with pathogenic fungi of *Fusarium spp.*, and noted German reports of a similar complex on *Quercus rubra* L. The beetle apparently vectors the fungus and provides the necessary wounds. The fungus kills the cambium of the tree causing a canker, and provides a suitable substrate for the beetle larvae. However, a 1993 survey by Carlson et al. of 183 black walnut plantations found only 1.6% of *Fusarium* cankers associated with attack by *Xylosandrus germanus*. It is capable of transmitting Dutch elm disease, and heavy infestations have been found on elms killed by this disease (Buchanan, 1941).

Xylosandrus zimmermanni (Hopkins) is probably native to Central and South America but, it is established in southern Florida on a wide variety of hosts (Wood, 1977). Baker (1972) states that it is established in New York City and the Ohio River Valley where it attacks hardwoods including elms, and is a possible vector for Dutch elm disease.

Platypodidae

Platypus solidus (Walker) is listed as a native of southern Asia and established in Japan. It has a wide range of host trees and was probably brought to Japan and possibly Borneo in logs (Marchant and Borden, 1976).

Platypus taiwansis (Schedl) is listed as a native of Taiwan which has become established in Japan where it attacks fig and oak trees in both countries. The method of introduction is unknown (Marchant and Borden, 1976).

HYMENOPTERA

Siricidae

Sirex noctillo (F.) is endemic to Eurasia and North Africa, where it is considered a secondary pest. It is primarily a pest of pines and has become established plantations of exotic pines in New Zealand (1900), Tasmania (1952), Australia (1961), and more recently in Argentina, Brazil and Uruguay (USDA, Forest Service, 1992). It was likely introduced on wood products since it has been intercepted in New South Wales in timber from Czechoslovakia (McMullin, 1953), and in Queensland in wood crates from Europe (Wylie and Yule, 1977). It is a major pest in Australia and South America. In Australia, it has caused up to 80% tree mortality in plantations over a three year period. In one year it and its fungal associate *Amylostereum aerolatum* Gaut killed 1.75 million trees in 56,400 hectares of plantations aged 10 to 30 (USDA, Forest Service, 1992). An earlier epidemic in New Zealand in the late 1940s killed an average of 30% of 120,000 hectares of trees in the 15 to 20 year age classes. This has been viewed in retrospect as a thinning, but the significant loss of increment cannot be ignored. Improved silvicultural and biological controls have reduced the severity of the problem in New Zealand (Forest Research Institute, 1974).

Sirex juvencus (L.) is an introduced species in eastern Canada and the northeastern and central United States; it is native to Europe. Baker (1972) lists its hosts as fir, pine and spruce. It was probably introduced on forest products and has been intercepted in Queensland on wood crates from Europe (Wylie and Yule, 1977).

Appendix B

Data of Refined Fumigation Tests

Armillaria ostoyae

Replicate Number	Target CxT mgh/l	Moisture Content %	Initial Ccn. mg/l	Final Ccn. mg/l	Wood Ccn. mg/l	Time hours	CxT mgh/l	Mort. %
Ao 240x72, 1	17064	117	237	45	820	72	10174	100
Ao 240x72, 2	17064	117	237	26	287	72	9464	100
Ao 240x72, 3	17064	117	237	23	622	72	9364	100
Ao 240x72, 4	17064	119	237	26	1183	72	9464	100
Ao 240x72, 5	17064	119	237	37	1184	72	9868	100
Ao 240x72, 6	17064	119	237	27	808	72	9497	100
Ao 240x24, 1	5688	144	237	42	1215	24	3344	100
Ao 240x24, 2	5688	144	237	41	1198	24	3338	100
Ao 240x24, 3	5688	144	237	27	653	24	3169	100
Ao 240x24, 4	5688	134	237	21	357	24	3097	100
Ao 240x24, 5	5688	134	237	46	1308	24	3402	100
Ao 240x24, 6	5688	134	237	48	1375	24	3422	100
Ao 240x 8, 1	1896	135	237	69	665	8	1226	100
Ao 240x 8, 2	1896	135	237	84	1346	8	1287	100
Ao 240x 8, 3	1896	135	237	69	591	8	1224	93.7
Ao 240x 8, 4	1896	116	237	82	1155	8	1275	100
Ao 240x 8, 5	1896	116	237	75	880	8	1249	100
Ao 240x 8, 6	1896	116	237	81	1306	8	1274	100
Ao 80x72, 1	5688	117	79	1	0	72	?	12.5
Ao 80x72, 2	5688	117	79	31	471	72	3971	100
Ao 80x72, 3	5688	117	79	1	0	72	?	0
Ao 80x72, 4	5688	119	79	23	383	72	3690	100
Ao 80x72, 5	5688	119	79	35	373	72	4104	100
Ao 80x72, 6	5688	119	79	32	375	72	4021	100
Ao 80x24, 1	1896	144	79	16	355	24	1145	100
Ao 80x24, 2	1896	144	79	34	608	24	1366	100
Ao 80x24, 3	1896	144	79	20	439	24	1186	100
Ao 80x24, 4	1896	134	79	21	242	24	1200	100
Ao 80x24, 5	1896	134	79	32	483	24	1336	100
Ao 80x24, 6	1896	134	79	28	316	24	1285	100
Ao 80x24, 7	1896	141	79	37	209	24	1391	100
Ao 80x24, 8	1896	141	79	41	383	24	1447	100
Ao 80x24, 9	1896	141	79	39	284	24	1421	100
Ao 80x24, 10	1896	135	79	0	0	24	?	0
Ao 80x24, 11	1896	135	79	32	119	24	1339	100

Armillaria ostoyae (continued)

Ao 80x24, 12	1896	135	79	26	145	24	1267	92.9
Ao 80x 8, 1	632	135	79	55	416	8	538	25
Ao 80x 8, 2	632	135	79	60	455	8	556	75
Ao 80x 8, 3	632	135	79	22	52	8	407	0
Ao 80x 8, 4	632	116	79	56	444	8	541	26.7
Ao 80x 8, 5	632	116	79	46	317	8	502	18.7
Ao 80x 8, 6	632	116	79	58	432	8	542	80
Ao 0x72, 1	0	117	0	0	0	72	0	6.2
Ao 0x72, 2	0	117	0	0	0	72	0	25
Ao 0x72, 3	0	117	0	0	0	72	0	18.7
Ao 0x72, 4	0	119	0	0	0	72	0	7.7
Ao 0x72, 5	0	119	0	0	0	72	0	6.2
Ao 0x72, 6	0	119	0	0	0	72	0	37.5
Ao 0x24, 1	0	146	0	0	0	24	0	33.3
Ao 0x24, 2	0	146	0	0	0	24	0	37.5
Ao 0x24, 3	0	146	0	0	0	24	0	91.7
Ao 0x24, 4	0	134	0	0	0	24	0	0
Ao 0x24, 5	0	134	0	0	0	24	0	6.7
Ao 0x24, 6	0	134	0	0	0	24	0	12.5
Ao 0x24, 7	0	141	0	0	0	24	0	31.2
Ao 0x24, 8	0	141	0	0	0	24	0	0
Ao 0x24, 9	0	141	0	0	0	24	0	31.2
Ao 0x24, 10	0	135	0	0	0	24	0	0
Ao 0x24, 11	0	135	0	0	0	24	0	43.7
Ao 0x24, 12	0	135	0	0	0	24	0	0
Ao 0x 8, 1	0	135	0	0	0	8	0	0
Ao 0x 8, 2	0	135	0	0	0	8	0	6.2
Ao 0x 8, 3	0	135	0	0	0	8	0	0
Ao 0x 8, 4	0	116	0	0	0	8	0	6.2
Ao 0x 8, 5	0	116	0	0	0	8	0	0
Ao 0x 8, 6	0	116	0	0	0	8	0	0

Heterobasidion annosum

Replicate Number	Target CxT mgh/l	Moisture Content %	Initial Ccn. mg/l	Final Ccn. mg/l	Wood Ccn. mg/l	Time hours	CxT mgh/l	Mort. %
Ha 240x72, 1	17064	104	237	23	210	72	9367	100
Ha 240x72, 2	17064	104	237	0	0	72	?	100
Ha 240x72, 3	17064	104	237	3	29	72	8647	100
Ha 240x72, 4	17064	134	237	15	146	72	9079	100
Ha 240x72, 5	17064	134	237	1	8	72	8543	100
Ha 240x72, 6	17064	134	237	29	250	72	9583	100
Ha 240x24, 1	5688	122	237	13	66	24	3006	91.7

Heterobasidion annosum (continued)

Ha 240x24, 2	5688	122	237	31	90	24	3221	100
Ha 240x24, 3	5688	122	237	43	268	24	3367	100
Ha 240x24, 4	5688	114	237	38	221	24	3301	100
Ha 240x24, 5	5688	114	237	35	182	24	3263	100
Ha 240x24, 6	5688	114	237	4	18	24	2892	100
Ha 240x24, 7	5688	136	237	129	795	24	4398	100
Ha 240x24, 8	5688	136	237	116	459	24	4263	100
Ha 240x24, 9	5688	136	237	139	1083	24	4512	100
Ha 240x24, 10	5688	136	237	110	448	24	4165	100
Ha 240x24, 11	5688	136	237	86	232	24	3872	100
Ha 240x24, 12	5688	136	237	102	347	24	4072	100
Ha 240x 8, 1	1896	122	237	73	1202	8	1239	95
Ha 240x 8, 2	1896	122	237	48	524	8	1142	100
Ha 240x 8, 3	1896	122	237	74	1276	8	1246	100
Ha 240x 8, 4	1896	129	237	50	653	8	1150	100
Ha 240x 8, 5	1896	129	237	46	512	8	1132	100
Ha 240x 8, 6	1896	129	237	72	1080	8	1235	100
Ha 80x72, 1	5688	104	79	8	11	72	3139	100
Ha 80x72, 2	5688	104	79	11	101	72	3251	100
Ha 80x72, 3	5688	104	79	0	0	72	?	12.5
Ha 80x72, 4	5688	134	79	10	100	72	3211	100
Ha 80x72, 5	5688	134	79	7	85	72	3121	100
Ha 80x72, 6	5688	134	79	0	0	72	?	C
Ha 80x24, 1	1896	122	79	26	115	24	1266	100
Ha 80x24, 2	1896	122	79	6	20	24	1020	38.5
Ha 80x24, 3	1896	122	79	11	59	24	1086	100
Ha 80x24, 4	1896	114	79	6	27	24	1013	C
Ha 80x24, 5	1896	114	79	6	49	24	1018	C
Ha 80x24, 6	1896	114	79	0	0	24	?	C
Ha 80x 8, 1	632	122	79	46	447	8	500	93.7
Ha 80x 8, 2	632	122	79	7	124	8	347	93.7
Ha 80x 8, 3	632	122	79	44	436	8	494	93.7
Ha 80x 8, 4	632	129	79	18	189	8	388	81.8
Ha 80x 8, 5	632	129	79	25	263	8	417	38.5
Ha 80x 8, 6	632	129	79	42	403	8	483	100
Ha 0x72, 1	0	104	0	0	0	72	0	31.2
Ha 0x72, 2	0	104	0	0	0	72	0	0
Ha 0x72, 3	0	104	0	0	0	72	0	0
Ha 0x72, 4	0	134	0	0	0	72	0	57.2
Ha 0x72, 5	0	134	0	0	0	72	0	18.7
Ha 0x72, 6	0	134	0	0	0	72	0	0
Ha 0x24, 1	0	122	0	0	0	24	0	31.2
Ha 0x24, 2	0	122	0	0	0	24	0	56.2
Ha 0x24, 3	0	122	0	0	0	24	0	0

Heterobasidion annosum (continued)

Ha 0x24, 4	0	114	0	0	0	24	0	C
Ha 0x24, 5	0	114	0	0	0	24	0	C
Ha 0x24, 6	0	114	0	0	0	24	0	C
Ha 0x24, 7	0	136	0	0	0	24	0	41.7
Ha 0x24, 8	0	136	0	0	0	24	0	83.3
Ha 0x24, 9	0	136	0	0	0	24	0	68.7
Ha 0x 8, 1	0	122	0	0	0	8	0	0
Ha 0x 8, 2	0	122	0	0	0	8	0	0
Ha 0x 8, 3	0	122	0	0	0	8	0	0
Ha 0x 8, 4	0	129	0	0	0	8	0	11.1
Ha 0x 8, 5	0	129	0	0	0	8	0	0
Ha 0x 8, 6	0	129	0	0	0	8	0	50

Lachnellula wilkommii

Replicate Number	Target CxT mgh/l	Moisture Content %	Initial Ccn. mg/l	Final Ccn. mg/l	Wood Ccn. mg/l	Time hours	CxT mgh/l	Mort. %
Lw 240x72, 1	17064	124	237	29	1149	72	9583	100
Lw 240x72, 2	17064	124	237	11	125	72	8928	100
Lw 240x72, 3	17064	124	237	23	850	72	9378	100
Lw 240x72, 4	17064	118	237	26	1261	72	9479	100
Lw 240x72, 5	17064	118	237	30	1260	72	9623	100
Lw 240x72, 6	17064	118	237	24	1355	72	9392	100
Lw 240x24, 1	5688	119	237	15	455	24	3025	100
Lw 240x24, 2	5688	119	237	2	148	24	2874	100
Lw 240x24, 3	5688	119	237	16	505	24	3035	100
Lw 240x24, 4	5688	117	237	9	511	24	2950	100
Lw 240x24, 5	5688	117	237	6	284	24	2915	100
Lw 240x24, 6	5688	117	237	11	509	24	2983	100
Lw 240x 8, 1	1896	125	237	51	762	8	1153	28.6
Lw 240x 8, 2	1896	125	237	68	1255	8	1220	100
Lw 240x 8, 3	1896	125	237	54	1251	8	1164	100
Lw 240x 8, 4	1896	133	237	58	1135	8	1180	93.3
Lw 240x 8, 5	1896	133	237	70	1439	8	1230	93.7
Lw 240x 8, 6	1896	133	237	69	724	8	1223	100
Lw 80x72, 1	5688	124	79	1	0	72	?	100
Lw 80x72, 2	5688	124	79	7	62	72	3118	100
Lw 80x72, 3	5688	124	79	5	65	72	3035	100
Lw 80x72, 4	5688	118	79	29	421	72	3906	100
Lw 80x72, 5	5688	118	79	17	235	72	3463	100
Lw 80x72, 6	5688	118	79	18	441	72	3488	100
Lw 80x24, 1	1896	119	79	0	90	24	?	100
Lw 80x24, 2	1896	119	79	11	175	24	1084	100

Lachnellula wilkommii (continued)

Lw 80x24, 3	1896	119	79	9	178	24	1056	100
Lw 80x24, 4	1896	117	79	6	161	24	1028	58.3
Lw 80x24, 5	1896	117	79	0	50	24	?	0
Lw 80x24, 6	1896	117	79	11	204	24	1085	100
Lw 80x 8, 1	632	125	79	17	347	8	386	0
Lw 80x 8, 2	632	125	79	9	336	8	353	0
Lw 80x 8, 3	632	125	79	12	333	8	366	12.5
Lw 80x 8, 4	632	133	79	9	340	8	354	0
Lw 80x 8, 5	632	133	79	13	438	8	370	0
Lw 80x 8, 6	632	133	79	22	534	8	405	0
Lw 0x72, 1	0	124	0	0	0	72	0	6.2
Lw 0x72, 2	0	124	0	0	0	72	0	0
Lw 0x72, 3	0	124	0	0	0	72	0	0
Lw 0x72, 4	0	118	0	0	0	72	0	0
Lw 0x72, 5	0	118	0	0	0	72	0	0
Lw 0x72, 6	0	118	0	0	0	72	0	0
Lw 0x24, 1	0	119	0	0	0	24	0	25
Lw 0x24, 2	0	119	0	0	0	24	0	6.2
Lw 0x24, 3	0	119	0	0	0	24	0	18.7
Lw 0x24, 4	0	117	0	0	0	24	0	25
Lw 0x24, 5	0	117	0	0	0	24	0	7.1
Lw 0x24, 6	0	117	0	0	0	24	0	6.2
Lw 0x 8, 1	0	125	0	0	0	8	0	0
Lw 0x 8, 2	0	125	0	0	0	8	0	0
Lw 0x 8, 3	0	125	0	0	0	8	0	0
Lw 0x 8, 4	0	133	0	0	0	8	0	0
Lw 0x 8, 5	0	133	0	0	0	8	0	0
Lw 0x 8, 6	0	133	0	0	0	8	0	0

Leptographium wageneri

Replicate Number	Target CxT mgh/l	Moisture Content %	Initial Ccn. mg/l	Final Ccn. mg/l	Wood Ccn. mg/l	Time hours	CxT mgh/l	Mort. %
Ow 240x72, 1	17064	117	237	33	263	72	9746	C
Ow 240x72, 2	17064	117	237	35	251	72	9796	C
Ow 240x72, 3	17064	117	237	13	88	72	8993	C
Ow 240x72, 4	17064	126	237	0	0	72	?	C
Ow 240x72, 5	17064	126	237	31	222	72	9760	C
Ow 240x72, 6	17064	126	237	44	416	72	10116	C
Ow 240x72, 7	17064	148	237	81	912	72	11448	100
Ow 240x72, 8	17064	148	237	81	882	72	11466	100
Ow 240x72, 9	17064	148	237	80	970	72	11434	100
Ow 240x24, 1	5688	116	237	78	1405	24	3784	100

Leptographium wageneri (continued)

Ow 240x24, 2	5688	116	237	60	509	24	3560	100
Ow 240x24, 3	5688	116	237	80	1353	24	3781	100
Ow 240x24, 4	5688	127	237	76	1284	24	3764	100
Ow 240x24, 5	5688	127	237	78	1470	24	3779	100
Ow 240x24, 6	5688	127	237	76	1214	24	3762	100
Ow 240x 8, 1	1896	109	237	80	1064	8	1267	100
Ow 240x 8, 2	1896	109	237	80	830	8	1270	100
Ow 240x 8, 3	1896	109	237	78	954	8	1262	100
Ow 240x 8, 4	1896	108	237	80	1060	8	1266	100
Ow 240x 8, 5	1896	108	237	80	1028	8	1268	100
Ow 240x 8, 6	1896	108	237	47	302	8	1138	43.7
Ow 80x72, 1	5688	117	79	0	219	72	?	C
Ow 80x72, 2	5688	117	79	14	284	72	3344	C
Ow 80x72, 3	5688	117	79	11	402	72	3236	C
Ow 80x72, 4	5688	126	79	10	6	72	3229	C
Ow 80x72, 5	5688	126	79	9	67	72	3179	C
Ow 80x72, 6	5688	126	79	1	91	72	2866	C
Ow 80x72, 7	5688	148	79	50	80	72	4658	100
Ow 80x72, 8	5688	148	79	53	71	72	4748	87.5
Ow 80x72, 9	5688	148	79	61	10	72	5036	100
Ow 80x24, 1	1896	116	79	58	361	24	1646	100
Ow 80x24, 2	1896	116	79	55	439	24	1613	100
Ow 80x24, 3	1896	116	79	50	364	24	1548	100
Ow 80x24, 4	1896	127	79	55	443	24	1615	100
Ow 80x24, 5	1896	127	79	52	318	24	1576	100
Ow 80x24, 6	1896	127	79	55	367	24	1616	100
Ow 80x 8, 1	632	109	79	62	444	8	565	7.1
Ow 80x 8, 2	632	109	79	63	452	8	570	0
Ow 80x 8, 3	632	109	79	63	454	8	569	12.5
Ow 80x 8, 4	632	108	79	65	396	8	579	6.2
Ow 80x 8, 5	632	108	79	65	376	8	576	6.2
Ow 80x 8, 6	632	108	79	59	381	8	554	25
Ow 0x72, 1	0	117	0	0	0	72	0	C
Ow 0x72, 2	0	117	0	0	0	72	0	C
Ow 0x72, 3	0	117	0	0	0	72	0	C
Ow 0x72, 4	0	126	0	0	0	72	0	C
Ow 0x72, 5	0	126	0	0	0	72	0	C
Ow 0x72, 6	0	126	0	0	0	72	0	C
Ow 0x72, 7	0	148	0	0	0	72	0	0
Ow 0x72, 8	0	148	0	0	0	72	0	50
Ow 0x72, 9	0	148	0	0	0	72	0	0
Ow 0x24, 1	0	116	0	0	0	24	0	25
Ow 0x24, 2	0	116	0	0	0	24	0	0
Ow 0x24, 3	0	116	0	0	0	24	0	0

Leptographium wageneri (continued)

Ow 0x24, 4	0	127	0	0	0	24	0	0
Ow 0x24, 5	0	127	0	0	0	24	0	0
Ow 0x24, 6	0	127	0	0	0	24	0	0
Ow 0x 8, 1	0	109	0	0	0	8	0	0
Ow 0x 8, 2	0	109	0	0	0	8	0	0
Ow 0x 8, 3	0	109	0	0	0	8	0	0
Ow 0x 8, 4	0	108	0	0	0	8	0	0
Ow 0x 8, 5	0	108	0	0	0	8	0	6.2
Ow 0x 8, 6	0	108	0	0	0	8	0	0

Phellinus weirii

Replicate Number	Target CxT mgh/l	Moisture Content %	Initial Ccn. mg/l	Final Ccn. mg/l	Wood Ccn. mg/l	Time hours	CxT mgh/l	Mort. %
Pw 240x72, 1	17064	131	237	74	452	72	11189	0
Pw 240x72, 2	17064	131	237	69	352	72	11041	0
Pw 240x72, 3	17064	131	237	87	996	72	11675	0
Pw 240x72, 4	17064	115	237	89	1323	72	11754	0
Pw 240x72, 5	17064	115	237	13	0	72	?	0
Pw 240x72, 6	17064	115	237	92	1700	72	11858	0
Pw 240x24, 1	5688	125	237	72	796	24	3704	C
Pw 240x24, 2	5688	125	237	82	912	24	3822	C
Pw 240x24, 3	5688	125	237	79	935	24	3791	C
Pw 240x24, 4	5688	122	237	77	750	24	3767	0
Pw 240x24, 5	5688	122	237	79	1087	24	3798	0
Pw 240x24, 6	5688	122	237	72	790	24	3708	0
Pw 240x 8, 1	1896	138	237	77	652	8	1259	C
Pw 240x 8, 2	1896	138	237	77	501	8	1256	C
Pw 240x 8, 3	1896	138	237	84	998	8	1284	C
Pw 240x 8, 4	1896	142	237	68	499	8	1222	C
Pw 240x 8, 5	1896	142	237	67	464	8	1215	C
Pw 240x 8, 6	1896	142	237	84	839	8	1284	C
Pw 80x72, 1	5688	131	79	36	96	72	4151	0
Pw 80x72, 2	5688	131	79	65	210	72	5188	0
Pw 80x72, 3	5688	131	79	56	200	72	4885	0
Pw 80x72, 4	5688	115	79	46	123	72	4522	0
Pw 80x72, 5	5688	115	79	63	206	72	5130	C
Pw 80x72, 6	5688	115	79	30	90	72	3924	C
Pw 80x24, 1	1896	125	79	44	315	24	1475	C
Pw 80x24, 2	1896	125	79	56	410	24	1620	C
Pw 80x24, 3	1896	125	79	60	491	24	1667	C

Phellinus weirii (continued)

Pw 80x24, 4	1896	122	79	47	256	24	1517	0
Pw 80x24, 5	1896	122	79	37	253	24	1391	C
Pw 80x24, 6	1896	122	79	27	123	24	1271	C
Pw 80x 8, 1	632	138	79	57	331	8	544	C
Pw 80x 8, 2	632	138	79	19	105	8	395	C
Pw 80x 8, 3	632	138	79	61	396	8	563	C
Pw 80x 8, 4	632	142	79	65	406	8	578	C
Pw 80x 8, 5	632	142	79	64	346	8	572	C
Pw 80x 8, 6	632	142	79	62	331	8	564	C
Pw 0x72, 1	0	131	0	0	0	72	0	C
Pw 0x72, 2	0	131	0	0	0	72	0	C
Pw 0x72, 3	0	131	0	0	0	72	0	C
Pw 0x72, 4	0	115	0	0	0	72	0	C
Pw 0x72, 5	0	115	0	0	0	72	0	C
Pw 0x72, 6	0	115	0	0	0	72	0	C
Pw 0x24, 1	0	125	0	0	0	24	0	C
Pw 0x24, 2	0	125	0	0	0	24	0	C
Pw 0x24, 3	0	125	0	0	0	24	0	C
Pw 0x24, 4	0	122	0	0	0	24	0	C
Pw 0x24, 5	0	122	0	0	0	24	0	C
Pw 0x24, 6	0	122	0	0	0	24	0	C
Pw 0x 8, 1	0	138	0	0	0	8	0	C
Pw 0x 8, 2	0	138	0	0	0	8	0	C
Pw 0x 8, 3	0	138	0	0	0	8	0	C
Pw 0x 8, 4	0	142	0	0	0	8	0	C
Pw 0x 8, 5	0	142	0	0	0	8	0	C
Pw 0x 8, 6	0	142	0	0	0	8	0	C

C = more than 50% of cube quarter sections contaminated
? = CxT values impossible to calculate due to unknown leakage

Appendix C

Analysis of Variance on the Effect of CxT Value on Mortality of All Species Tested in Refined Studies

Dependant Variable: Log Mortality					
Source	DF	Sum of Squares	Mean Square	F Value	P Value
Model	124	39.6287	0.3196	589.23	0.0001
Error	5	0.0027	0.0005		
Corrected Total	129	39.6314			
	R-square	C.V.	Root MSE	Log Mortality Mean	
	0.9999	-12.5549	0.0233		-0.1855
Source	DF	Type I SS	Mean Square	F Value	P Value
Log(CxT)	124	39.6287	0.3196	589.23	0.0001
Source	DF	Type III SS	Mean Square	F Value	P Value
Log(CxT)	124	39.6287	0.3196	589.23	0.0001