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Dr. Jeffrey J. Morrell

Metham sodium or NaMDC is one of the four major soil fumigants that are currently registered for controlling internal decay of wood products in use. Fumigants are used primarily in utility poles but are also applied to piling, timbers and other large wood members.

NaMDC must decompose to fungitoxic compounds to become effective. A variety of volatile and non-volatile decomposition products are produced during NaMDC breakdown in wood but the role these components play in the overall fungitoxicity of metham sodium is poorly understood. Volatile methylisothiocyanate (MITC) is believed to be the primary fungitoxic NaMDC breakdown product but it has been suggested in previous studies that some synergistic activity between MITC and other volatile NaMDC decomposition products in wood might occur, resulting in an enhanced MITC fungitoxicity.

A fumigation apparatus was designed to examine the toxicity of two NAMDC volatile decomposition products,

carbon disulfide and methylisothiocyanate, against six decay fungi (basidiomycetes) and one mold (ascomycete) established in Douglas-fir and Ponderosa pine. Fumigations were performed with the individual compounds at different concentrations for a period of ten days to determine sublethal dosages of each chemical. Fumigant effects were assessed by grinding blocks and plating a portion of the ground material in malt extract agar. The resulting number of colony forming units (CFU's) provided a measure fungal survival. A stimulus in the number of CFU's was observed for most species treated with lower fumigant concentrations suggesting that some fungi had the ability to metabolize sulfur-containing fumigants when growing in a low sulfur-containing environment. Large variations in number of CFU's were exhibited among the fungi but CT_{90} values showed that the CS_2/MITC mixture was more fungitoxic to most fungi than fumigations with the individual fumigants.

The results indicate that NaMDC decomposition products can interact synergistically to enhance fungal control in wood. The effect may help to explain the effectiveness of NaMDC as a wood fumigant despite its relatively low MITC yield. Further studies using other decomposition products would help to better define other interactions in this process.

Fungitoxicities of NaMDC Decomposition Products to Decay Fungi Colonizing Douglas-fir and Ponderosa Pine Wood

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Signature redacted for privacy.

Dr. Jeffrey G. Morrell
Professor in the Department of Forest Products

Signature redacted for privacy.

Dr. Thomas E. McLain
Head of Department of Forest Products

Signature redacted for privacy.

Dean of Graduate School

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Typed by Edwin Canessa

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I

INTRODUCTION

Wood has been utilized by man as raw material since ancient times for a large variety of products. As an organic material, wood is susceptible of decay by microorganisms, mainly fungi, that use it as a source of energy. Wood species have differing natural durability against decay organisms. Many wood species have naturally durable heartwood, while others have moderately or non-decay resistant heartwood.

The need to improve the use of wood as a material encouraged the development of the wood preservation industry. Preservation of wood first started by elevating wood on stones to exclude moisture or by daubing wood in oil of cedar to prevent its decay (Graham 1973). Many different preservation methods have been used and these processes have become an increasingly important industry in many countries.

Wood preservation in the United States has been one of the fastest growing segments of the forest products industry, especially during the past decade (Micklewright, 1990). Preservation not only extends the useful life of the material, but also extends the forest resource, decreasing the need to harvest timber to replace wood which has failed due to decay.

As a general rule, the sapwood of all species has little resistance to decay (Panshin and DeZeeuw, 1980; Scheffer, 1973). Wood preservation protects this material by impregnating it with toxic substances or by altering wood-moisture relationships making the wood unrecognizable by a fungus (Graham, 1983). The chosen method and type of preservative, as well as penetration and retention, depend on wood species and final use of the product.

Heartwood of most species is resistant to preservative treatment and certain species like Douglas-Fir (*Pseudotsuga mensiezii* [Mirb.] Franco.) have a particularly impermeable heartwood surrounded by a very thin band of sapwood (Graham, 1961; Miller, 1961). As a result, preservative treatment produces a thin envelope which provides suitable protection, given that the chemical barrier is not compromised (Morrell, 1989). Some products, especially those with large dimensions such as poles and ties, cannot be economically dried to moisture contents they will attain in service (Graham, 1983). As they dry in service, severe checking may develop. Some of these checks may expose untreated heartwood to possible attack by wood destroying organisms. Inspections of Douglas-fir distribution and transmission poles in Western Oregon showed that the treated sapwood was sound, but internal decay was a serious problem (Graham, 1973). *Postia placenta* and *Antrodia carbonica* were the decay fungi most frequently associated with internal decay in these poles (Graham and Corden, 1980).

A variety of methods have been used to prevent and stop internal decay in poles and piles. The Cobra process, which was originally patented in 1922, was one of the first

attempts to achieve deep penetration of sodium fluoride paste in poles in service (Smith and Cockcroft, 1967a, 1967b, 1967c; Graham, 1973). This system is widely used in Europe, but has seen little application in North America. Attempts were also made to use external bandages or to drill holes and pour oil based insecticides into the holes; however, these treatments lacked the ability to migrate for significant distances into the wood and were largely ineffective. In the late 1960's, Bonneville Power Administration and Oregon State University evaluated the application of fumigants which had previously been used for agricultural soil treatments (Hand et al, 1970; Ricard et al., 1968; Helsing et al., 1984; Morrell and Corden, 1986). The results were promising and a number of utilities incorporated these treatments into their maintenance programs. Fumigants are now widely used by electric utilities to arrest internal decay in wood products (Goodell and Graham, 1983). At present, four fumigants, Vapam (32.7% sodium methyl dithiocarbamate), chloropicrin (96% trichloronitromethane), Vorlex (20% methylisothiocyanate in chlorinated C_3 hydrocarbons) and MITC-Fume (96% methylisothiocyanate), have been registered for application to wood products. Vapam (metham sodium) and chloropicrin are the two most commonly used chemicals, with the former being used for poles in urban areas and the latter being used for application to poles in rural areas (Morrell, 1989).

Several studies have evaluated chloropicrin and MITC movement and toxicity within the wood (Cooper et al., 1974; Goodell et al, 1980; Zahora, 1987; Zahora and Morrell, 1989 a,b; Zahora and Corden, 1985), but there are few studies on toxicity of metham sodium (NaMDC) in wood. NaMDC must

decompose to MITC to become effective and does so at a theoretical 40% efficiency rate. A variety of other decomposition products are produced, but the role of these components are poorly understood (Miller and Morrell, 1990; Morrell and Lebow, 1989; Graham, 1973; Scheffer and Graham, 1975; Corden et al., 1988a). Despite the relatively low efficiency of metham sodium decomposition in wood, this chemical is reasonably effective, providing 7 to 10 years of protection to Douglas-fir poles (Helsing et al., 1984). The improved performance may reflect interactions between various decomposition products to enhance MITC activity; however this subject has received little attention.

The purpose of this study was to examine the role of carbon disulfide, an NaMDC decomposition product, alone or in combination with MITC on toxicity against molds or decay fungi established in Douglas-fir and Ponderosa pine.

II
LITERATURE REVIEW

2.1 Use of fumigants in soil

The effectiveness of fumigants against soil fungi is well documented (Newhall, 1955; Thorn and Ludwig, 1962). Soil fumigants are organic chemicals with either a high vapor pressure or a low boiling point, necessitating that they be kept under pressure when not in use. Alternatively, liquid can be absorbed on some inert powder which acts as a carrier (Parrish, 1958). Carbon disulfide was the most widely used fumigant until 1945 and was the first fumigant which was injected into the soil to control the grape *Phylloxera* in France and Germany (Newhall, 1955).

Large stocks of toxic gases were produced during World War I for use as chemical weapons. Trichloronitromethane, also known as chloropicrin or tear gas, was known to have general biocidal properties, but it was not until the early 30's that it was used as a general soil fumigant (Johnson and Godfrey, 1932).

Another important soil fumigant is methyl bromide which has been used for control of weeds in nurseries (Molin and Tear, 1956). Methyl bromide has also been used for fumigation of granaries, packed foodstuffs, and fresh fruits to control insect infestations (Parrish, 1958). Methyl bromide is an odorless and very toxic gas which is difficult to handle due to its low boiling point (40° F). Chloropicrin (2%) usually added to serve as an indicator for the presence of this poisonous gas (Parrish, 1958).

Another important group of soil fumigants are the carbamate derivatives. According to McCallan (1967), in 1931 Tisdale and Williams, assignors to E. I. du Pont & Co., applied for a patent on derivatives of dithiocarbamic acid as disinfectants to control and prevent the growth of fungi and microbes. Dithiocarbamates were used as accelerators in the rubber industry until 1934 when a U.S. patent was granted for their use as fungicides. Thiram, ferbam and ziram were among the first generation of dithiocarbamates fungicides (McCallan, 1967). Sodium N-methyl-dithiocarbamate or NaMDC, commercially known as metham sodium, is also a derivative of dithiocarbamic acid. Introduced to the market in 1955 (Parrish, 1958), metham sodium is usually applied as a 32.7% aqueous solution which produces volatile fungitoxic products as it decomposes. Metham sodium is used to control soil borne plant pathogenic fungi, nematodes, insects and weed seeds (Foster, 1956; Briscoe and Strickland, 1956; Elson, 1966).

2.2 Use of fumigants in wood

The use of soil fumigants to arrest internal decay in wood structures such as poles and piling has become a common practice among utilities throughout the United States (Goodell and Graham, 1983).

Kuntz and Drake (1960) first used methyl bromide to kill oak roots, to prevent the local spread of the wilt fungi, *Ceratocystis fagacearum* between root grafts. It was not until Partridge (1961), while evaluating chemicals for control of the oak wilt fungus, demonstrated that some fungicides were capable of moving through heartwood, that

fumigation received serious consideration. Patridge demonstrated that methyl bromide and chloropicrin vapors were capable of moving through the wood at concentrations sufficient to kill the oak wilt fungus *Ceratocystis fagacearum*, in oak heartwood. Later, Jones (1963) confirmed the ability of methyl bromide to eliminate the oak wilt fungus from oak logs. When applied to wood, little or no trace of methyl bromide remains after treatment, permitting rapid reinfestation by decay fungi (Morrell, 1989). These disadvantages made fumigation of large wood members with this fumigant impractical.

Chloropicrin, is also highly volatile, but can be detected easily because it is a strong irritant. It has the ability to bind effectively to sound wood, possibly due to reactions with lignin (Goodell et al., 1985). Chloropicrin appears to interact strongly with wood, where it can be detected up to 20 years after treatment of Douglas-fir poles. This interaction may account for the long term protection provided by the chemical (Goodell et al., 1985; Morrell, 1989). Chloropicrin is one of the two most commonly used chemicals for fumigant treatment of wood poles. Although chloropicrin is a very efficient fumigant, its high volatility has led many utilities to use other products with improved handling safety.

Metham sodium has some advantages over other fumigants. Its low volatility makes it easy to handle, although it is caustic and may irritate the eyes or skin. Metham sodium has minimal fungitoxic activity and must decompose to become fungitoxic. One of the primary decomposition products is methylisothiocyanate (MITC), which is volatile and highly fungitoxic. At least 4 other

volatile compounds can be produced from commercial metham sodium (Morrell, 1989; Miller and Morrell, 1990). Solid NaMDC is relatively stable in air while NaMDC in solution oxidizes readily. According to Graham and Corden (1980), aqueous solution formulations may decompose more rapidly in wood making them effective in wood than pure NaMDC. Solid NaMDC as pure salt, can be applied safely as a powder or in pellets, but the absence of water slows the decomposition rate (Morrell et al., 1988). Adding water to metham sodium treated wood increased MITC yield from between 30% and 45% to 60 and 65% (Miller and Morrell, 1990).

In addition to the volatile chemicals produced during metham sodium decomposition, there is evidence of deposition of up to 10 non-volatile components including elemental sulfur and dimethylthiuram disulfide some distance from the point of application (Hand, et al., 1970; Miller and Morrell, 1990). Although these materials may contribute to the long term protection against reinfestation shown by the NaMDC, their role is poorly understood.

2.3 Decomposition products of metham sodium in soil

Metham sodium is a relatively non-selective soil fumigant whose fungitoxic action is generally believed to be due to its decomposition to MITC, although additional decomposition products are present as a result of secondary reaction products (Turner, 1962). Although the fungitoxicity of most known or potential decomposition products of NaMDC has been investigated (Elson, 1966), these studies were performed using individual components.

In his research, Elson concluded that MITC must have a different mode of fungicidal action than metham sodium, and suggested that there may be some interaction between MITC and other metham sodium decomposition products.

Decomposition of metham sodium in soil (Table 1) is affected by a number of factors including moisture content, temperature and pH (Turner and Corden, 1963; Goring, 1967; Smelt and Liestra, 1974; Zahora, 1983). Turner (1962), found that 70 to 87 per cent of the metham sodium applied to soil was accounted for as MITC, under various treatment conditions. Turner and Corden (1963) found that lower levels of MITC were produced from metham sodium in soil with a nitrogen atmosphere than in soil with air.

Table 1. Possible NaMDC decomposition products in soil[a]

CAS name	Abbreviation
Methylisothiocyanate	**(MITC)**
N,N'-dymethylthiuram disulfide	**(DMTD)**
2,4-dimethyl-1,2,4-thiadiazolidine-3,5-dithione	**(DTD)**
4-methyl-5-(methylimino)-1,2,4-dithiazolidine-3-thione	**(MMDT)**
Sodium trithiocarbonate	**(NaTTC)**
Carbon disulfide	**(CS2)**
Carbonyl sulfide	**(COS)**
Hydrogen sulfide	**(H2S)**
Methylamine	
N,N'-dimethylthiourea	**(DMTU)**
Sulfur	**(Sulfur)**

[a] From Turner, 1962; Elson, 1966; Miller and Morrell, 1990

Under basic conditions, aqueous metham sodium decomposes primarily to MITC and elemental sulfur while carbon disulfide, hydrogen sulfide, dimethylthiuram

disulfide (DMTD), methylamine and MITC are produced under acid regimes (Thorn and Ludwig, 1963).

Some decomposition products can undergo further reactions to produce other compounds. Methylamine, CS_2 and H_2S may be especially important in the synthesis of such compounds. For example, CS_2 and methylamine can react to form MITC which, in turn, can react with metham sodium to yield DMTD and with methylamine or H_2S to produce DMTU (Turner, 1962; Turner and Corden, 1963). Turner and Corden (1963) detected more DMTU after metham sodium application to sandy loam soil than before it was added to the soil, suggesting that reactions mentioned previously occurred.

2.4 Decomposition products of metham sodium in wood

Metham sodium appears to decompose in wood in much the same manner as it decomposes in soil (Table 2), primarily producing methylisothiocyanate.

Graham and Corden (1980) found that metham sodium breakdown in wood released a volatile compound which was probably MITC, although the efficiency of the process in an acid environment such as wood, was not determined. MITC continues to decompose in a series of reactions to produce carbon disulfide (CS_2), carbonyl sulfide (**COS**), dimethythiourea (**DMTU**), dimethyl thiadiazolidine dimethione (**DTD**) and elemental sulfur. The concentrations of these decomposition products depend on the moisture content and probably also on temperature of wood at the time of fumigant application (Zahora and Morrell, 1988)

Table 2. Non volatile and volatile decomposition products of NaMDC in wood.[a/]

CAS name	Abbreviation
Non volatile	
Sodium thiosulfate	
Sodium sulfate	
N,N'-dimethylthiourea	(DMTU)
O-methyl-N-methylcarbamothioate	(MMC)
2,4-dimethyl-1,2,4-thiadiazolodone-3,5-dithione	(DTD)
4-methyl-5-(methylimino)-1,2,4-dithiazolidine-3-thione	(MMDT)
Sulfur	Sulfur
Volatile	
Isothiocyanato-methane	(MIT)
Carbonyl sulfide	(COS)
Carbon disulfide	(CS2)
Hydrogen sulfide	(H2S)
Methylamine	

[a/] From Miller and Morrell, 1990.

2.5 Fungitoxicity of decomposition products.

Elson (1966), studied fungitoxicity of metham sodium decomposition products against *Fusarium oxysporum* spores. Dimethylthiuram disulfide (DMTD) was highly fungitoxic to spores but along with NaTTC, DTD and NMDC, could not completely account for the effectiveness of metham sodium. None of these compounds were sufficiently volatile to account for the toxic effect noted some distance from the point of application nor were the fungitoxicities of individual decomposition products such as H_2S, COS, CS_2, DMTU and methylamine sufficient to account for metham sodium effectiveness. Miller et al. (1953) found that very large amounts of hydrogen sulfide (10,000 to 60,000 ppm. on

a spore weight basis) could be formed by spores of nine species of fungi exposed to elemental sulfur without completely inhibiting germination and concluded that H_2S was not very toxic to fungus spores under the conditions evaluated.

Zahora and Corden (1985) determined the effectiveness of MITC against *Antrodia carbonica* in Douglas-fir heartwood exposed to various fumigation periods. The product of MITC concentrations and exposure times required to kill 90% (CT_{90}) of the fungal propagules ranged from 46 to 179 µg hr/cc air. These values were influenced by moisture content of wood and duration of fumigant exposure. At constant, low MITC vapor concentrations (less than 1 ug/cc air), wood at a low moisture content (10%) adsorbed 5 times more MITC, but required 4 times the exposure period to control *A. carbonica*, than wood above fiber saturation point (fsp). Increased susceptibility was apparently dependent on the water content of the mycelium (Zahora and Morrell, 1989b). There seems to be no significant difference in MITC fungitoxicity at different moisture contents above the fsp. (Zahora and Corden, 1985). These studies suggest that MITC is the primary fungitoxic component of metham sodium decomposition; however, the presence of relatively high levels of other decomposition products may also influence fumigant performance.

Carbon disulfide is a common metham sodium breakdown product in wood (Miller and Morrell, 1990). The use of carbon disulfide as a partial soil sterilant was first recorded at the end of last century when it was injected into soil to control the grape *Phylloxera* (Newhall, 1955). Since then, carbon disulfide has been reported to have

biocidal effects on *Armillaria mellea* (Blis, 1951; Filip and Roth, 1977; Munnecke et al., 1973), *Trichoderma viride* (Munnecke et al., 1973), *Clitoscibe tabescens* and a variety of plant parasitic nematodes.

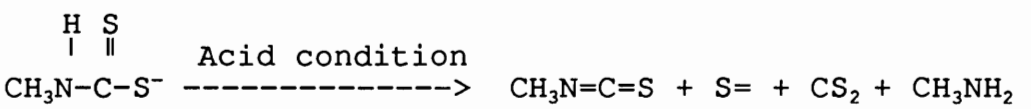
Although fungitoxicity of carbon disulfide has been recognized, the literature on toxic dosages is contradictory. Garret (1957), reported that fumigation with 207 ppm of CS_2 for 72 hours was sufficient to kill *A. mellea* in small inoculum segments by direct fungicidal action. Conversely, Munnecke et al. (1973) reported that fumigations of *A. mellea* with 6,000 ppm carbon disulfide for up to 18 days and *T. viride* with 3,180 ppm carbon disulfide for 2 to 10 days stopped mycelium radial growth but did not kill the fungi.

Sodium tetrathiocarbonate releases carbon disulfide when added to water and applied to soils. Applications of 2,450 ug/ml sodium tetrathiocarbonate (equivalent to approximately 1000 ug/ml carbon disulfide) for a period of 72 hours where needed to completely inhibit mycelial growth in six species of *Phytophtora* (Matheron and Matejka, 1988). Zoospores cysts formed after 4 to 6 hours exposure in the presence of 2.4 ug/ml of this compound (0.8 ug/ml carbon disulfide) were viable, whereas those exposed to 12 ug/ml (5 ug/ml carbon disulfide) failed to germinate.

Metham sodium, and in fact most carbamates, decompose to produce carbon disulfide under acidic conditions (Thorn and Ludwig, 1963). Acidic conditions are prevalent in wood from temperate zones which have pH value range from 3 to 6. Douglas-fir, the species most often fumigant-treated has a pH of about 3.3 (Fengel and Wagener, 1984). This favors

formation of carbon disulfide as a breakdown product of metham sodium in wood.

Theoretically, decomposition of metham sodium under low pH conditions could yield up to one carbon disulfide per metham sodium. Controlled decomposition is a primary method for determining dithiocarbamate residue in agricultural products. MITC is also produced when metham sodium is applied to wood (Miller and Morrell, 1990). The formation of MITC and CS_2 as decomposition products of metham sodium under acidic conditions may occur through the following reaction (Turner and Corden, 1963):



$$CH_3N(H)-C(=S)-S^- \xrightarrow{\text{Acid condition}} CH_3N=C=S + S= + CS_2 + CH_3NH_2$$

Since both CS_2 and MITC, along with other decomposition products, are present at a given time within metham sodium fumigated wood, some synergistic reactions may occur among the potential NaMDC decomposition products may occur. Decomposition in soil is complicated by the presence of a variety of organic and inorganic materials. Wood is far less reactive and is therefore, simpler to study. Most of the studies on metham sodium fungitoxicity have been performed on wood treated with only one decomposition product and they cannot completely account for the fungitoxicity of NaMDC. Developing an improved understanding of the interactive effects of metham sodium decomposition products may provide clues concerning activity under various conditions and identify strategies for improving chemical performance. The present study

represents an effort to better understanding the fungitoxicity of two metham sodium decomposition products in wood.

III

METHODOLOGY

Blocks colonized by 6 basidiomycetes and 1 ascomycete were prepared using modifications of a previously described procedure (Sexton et al., in press) (Table 3).

Table 3. List of test fungi evaluated for sensitivity to carbon disulfide and MITC.

Name	Isolate#	Source
Antrodia carbonica(Overh.)Ryv&Gilbn.	L-8242-sp	FRL.Corvallis OR
Postia placenta (Fr.)M. Lars & Lomb.	MAD - 698	FPL.Madison WI.
Irpex lacteus (Fr.:Fr.) Fr.	FP-105915-sp	FPL.Madison WI.
Trametes versicolor (L.:Fr.) Pilát.	A4CD-34	FRL.Corvallis OR
Hormoconis resinae v. Arx & de Vries	P1600	SUNY College of Env. Sci. & For.
Gloeophyllum trabeum (Fr.) Murr.	MAD-617	FPL.Madison WI.
G. saepiarium (Fr.) Karst	S4UT	FRL.Corvallis OR

Ponderosa pine sapwood (*Pinus ponderosa* Laws) and Douglas-fir heartwood (*Pseudosuga menziesii* (Mirb) Franco) blocks (10 by 10 by 3 mm. long) were placed in autoclavable plastic bags equipped with a single breathable patch. Approximately 100 g of vermiculite fine grain and 700 ml of distilled water were added to the bags which were loosely sealed prior to autoclaving for 20 min at 120 C. The bags were then inoculated with a macerated hyphal/spore mixture of the respective fungus. Basidiomycete inoculum was prepared by placing a small agar plug cut from the edge of an actively growing malt extract agar culture of the test fungus into a flask containing 50 ml of 1.8 % malt extract solution. The flasks were incubated at room temperature on

a rotary shaker (80 rpm) for 7 to 14 days, then the mycelium was filtered, resuspended in sterile distilled water and blended for 10 seconds at approximately 11,000 rpm. The macerated mycelium mixture from a single flask was transferred to a squeezable bottle and poured over the blocks in the plastic bags. The bags were heat sealed and incubated 20 to 30 days at 27 C. *Hormoconis resinae* was grown on 1.8% malt extract agar and the plates were flooded with sterile distilled water to dislodge conidia. The conidia were poured over the blocks in the same manner as the mycelial suspension. Colonization during the incubation period was periodically assessed by removing selected blocks from the bags and placing them on malt extract agar. Growth of the test fungus from the blocks was used as a measure of successful colonization.

The blocks were exposed to metham sodium decomposition products in a fumigation apparatus consisting of a series of five 40 ml wide mouth glass jars each capable of holding a different metham sodium decomposition product (Fig. 1). The jars were equipped with Teflon® lined caps to retard possible fumigant loss. Teflon® tubing (6 mm outer diameter) was used to connect the jars so that different ratios of the selected fumigants could be introduced into the system. The five bottles were in turn connected to a single mixing vessel which was connected to a manifold which distributed the gas mixture to a series of twelve 135 ml glass jars. Each jar contained 10 blocks colonized by a single fungus. Flow from the fumigant jars to the mixing chamber was controlled using Teflon® line control valves, while flow to individual fumigation chambers was controlled using glass restrictor tubes packed with Celite® (diatomaceous earth) to produce a flow of 15 ml fumigant

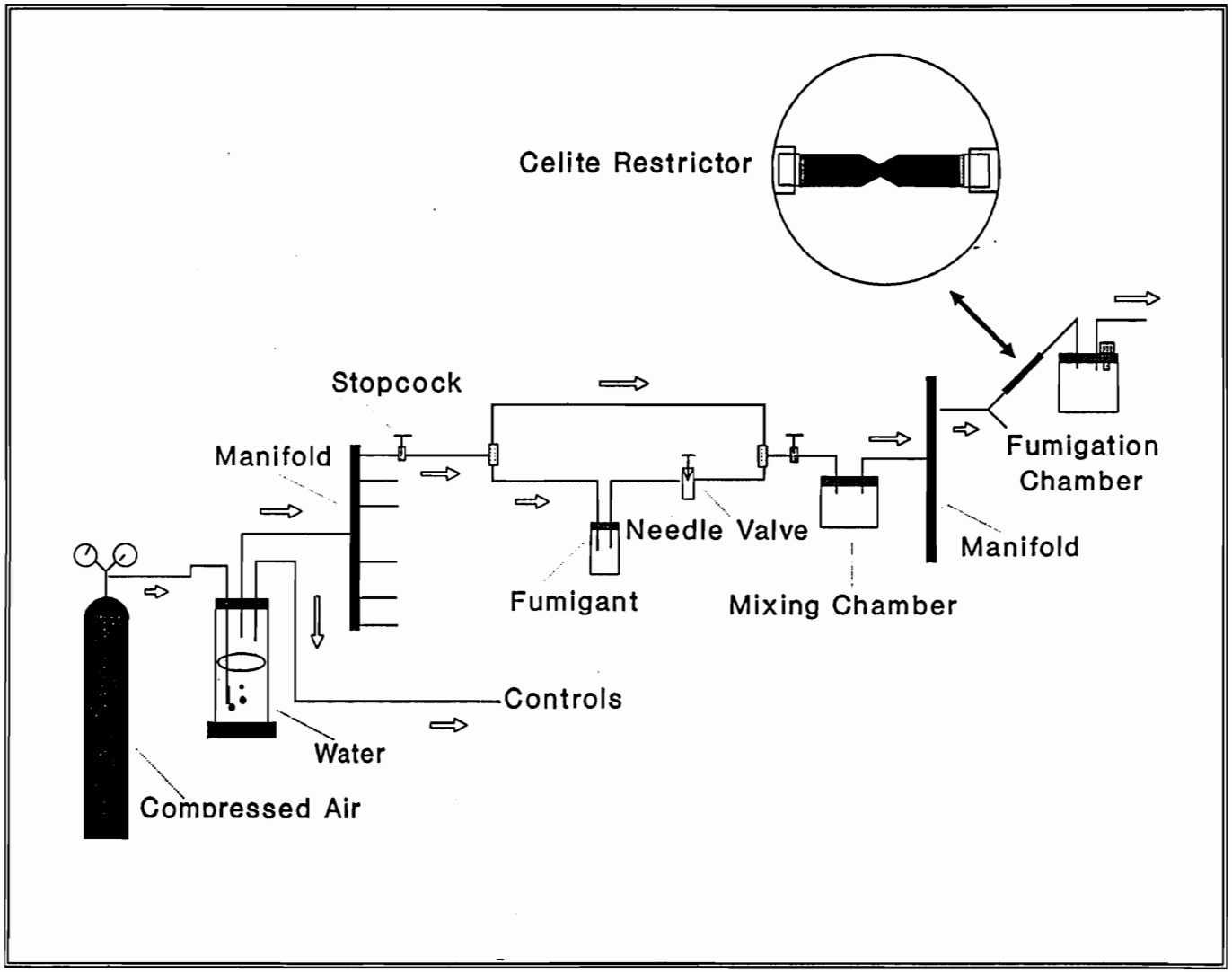


Fig.1 -Apparatus employed to fumigate fungus colonized wood blocks.

laden air/minute. All gas flows were measured using a bubble flow meter. Fumigant concentrations were varied by increasing the flow of gas from a given fumigant component reservoir to the mixing vessel.

Air flowing through the apparatus was first humidified by bubbling through distilled water, then the air flowed over the jars containing the fumigant, through the mixing jars and finally into the jars containing the fungal colonized blocks. Fumigant flow rates were adjusted until the desired concentration of each gas was achieved, then the apparatus was allowed to operate for 24 hours prior to introduction of fungal colonized blocks.

Fumigant concentrations over the course of the trial were assessed by removing air samples from a site on the fumigation chamber in which a rubber serum cap had been inserted. For carbon disulfide, 5 ul of gas was injected into a Variant 3700 Gas Chromatograph equipped wit a Flame photometric detector with filters specific for sulfur. The GC conditions were as follows: nitrogen flow rate 33.3 cc/minute; detector temperature 240 ºC; injector temperature 150 ºC and column temperature was at 40 ºC. Separation was achieved using a 3 m long by 4 mm inner diameter column packed with 10 % Carbowax 20M On 80/100 Supelcoport (Supelco, Bellafonte P.A). For methylisothiocyanate, 200 ul air samples were injected. GC conditions were similar except that column temperature was increased to 110 ºC.

The fumigant chamber was first employed to evaluate the fungitoxicity of 0.5, 3-4 and 8-9 ng of carbon disulfide per ul of air or 5, 10, and 18 ng MITC/ ml of

air. The results from these trials indicated that 3 to 4 ng of carbon disulfide and 5 ng MITC were sublethal exposures rates and the effects of a mixture of these two gases at the sublethal level was then evaluated.

Fumigations were carried out over 10 day periods. Each day, one block colonized by each fungus species was removed from each chamber and aerated to permit the fumigant to dissipate. The aerated block was placed into a 36 ml stainless steel canister containing stainless steel ball and 5 ml of sterile distilled water. The canister was shaken for 5 seconds using a Kleco® 4100 Pulverizer (Kleco Kenetic Manufacturing Co., Visalia, Ca). After maceration, the steel ball was removed using a magnetic stir bar wand, and the macerated wood suspension was diluted to 30 ml with additional distilled water. A 1.5 ml aliquot of the suspension was added to 10 ml of molten (45 ºC) 1.8 % malt extract agar and the mixture was poured into a petri dish and allowed to solidify. Three plates were prepared from each macerated block. The plates were incubated at room temperature and the number of colonies which developed in each plate were counted. These results were compared with colony counts of similar blocks which were not exposed to the fumigants.

The data were used to construct concentration x time (CT) curves to assess the amount of chemical necessary to kill each fungus at selected exposures. In addition, the mean number of colony forming units at selected time points were subject to an Analysis of Variance (ANOVA) and the means were compared using LSD at alpha= 0.05.

IV
Results and Discussion

4.1 Fungitoxicity of carbon disulfide.

Table 4 summarizes the average percentage survival (based on control counts) of seven species of fungi on each of the two species of wood following fumigation with carbon disulfide. The number of CFU's (colony forming units) varied widely between the fungal species, reflecting both the sensitivity of each species to the isolation procedures as well as the presence or absence and relative amounts of conidia or chlamydospores in the wood. *Hormoconis resinae* produced the largest number of CFU's reflecting the massive sporulation which is a common feature of this fungus (Bessey, 1950). *Irpex lacteus* produced the smallest number of CFU's and showed the highest sensitivity to both fumigants. The absence of asexual spores or other special survival structures and the presence of thin to slightly thickened generative hyphae help explain the sensitivity of this fungus (Wang and Zabel, 1990).

In addition to the variation between fungal species, CFU's in some non-fumigant exposed control blocks tended to decline slightly over the 10 day test period. This effect was more noticeable in *I. lacteus* than in any other species. Efforts were made to humidify the atmosphere by bubbling the air used in the test apparatus in distilled water before it passed over the blocks. Water condensed on the walls of the tubing along the line, even in areas as close to the fumigation chambers as flow restrictors as well as in connections between fumigation lines and

Table 4.- Effect of fumigation with CS_2 on ability to produce colony forming units (CFU's) for seven species of fungi on pine or Douglas-fir as a % of controls. [a/]

Time (days)	*A. carbonica*		P. placenta		L. lacteus		G. trabeum		G. saeplarium		T. versicolor		H. resinae	
	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine
	500 ppm. carbon disulfide.													
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	107	90	212	152	183	105	94	93	147	104	286	230	96	163
2	108	88	263	174	153	114	106	136	148	107	257	195	101	153
3	107	109	199	144	104	101	93	101	140	108	323	183	92	158
4	101	114	255	130	139	127	107	103	172	121	272	233	104	148
5	109	153	162	182	144	90	107	126	144	102	324	297	109	154
6	131	186	168	172	86	101	93	140	152	116	225	183	122	145
7	99	138	110	153	111	117	88	107	124	136	332	367	137	150
8	99	107	122	117	96	133	95	88	143	108	328	226	126	151
9	114	93	140	133	122	115	100	77	133	105	333	182	143	162
10	105	93	140	156	98	107	107	70	138	129	317	278	127	160
	3000 to 4000 ppm. carbon disulfide.													
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	92	102	232	194	176	39	203	291	107	133	255	130	86	127
2	101	101	286	288	119	51	173	330	106	186	369	131	93	133
3	112	120	177	180	66	7	200	300	78	113	57	183	84	101
4	97	65	125	90	52	6	142	53	68	66	55	180	81	102
5	118	43	94	45	43	2	118	31	98	66	107	122	91	91
6	128	29	91	17	25	2	95	37	57	72	104	89	111	86
7	103	17	71	7	14	0	106	22	78	108	54	144	88	76
8	81	16	57	4	3	0	134	19	66	68	76	84	83	67
9	55	14	49	3	1	0	126	14	69	64	89	69	73	69
10	47	15	55	2	0	0	103	14	71	51	79	27	69	66
	8000 to 9000 ppm. carbon disulfide													
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	122	150	420	370	48	109	90	32	128	114	40	225	74	70
2	117	148	363	191	58	150	76	81	98	137	45	171	72	74
3	87	130	333	152	25	75	76	107	85	143	72	126	57	56
4	70	84	180	92	23	34	98	121	66	110	69	294	50	41
5	83	67	152	42	3	3	122	49	43	68	66	168	55	38
6	64	25	90	16	3	1	90	30	24	86	50	328	57	34
7	71	8	62	6	2	0	61	13	5	55	45	91	52	31
8	47	3	42	3	0	0	46	16	4	38	25	74	49	29
9	32	1	5	1	0	0	4	3	2	30	5	24	46	21
10	30	0	1	0	0	0	1	0	1	21	1	16	33	19

[a/]Values represent the average of 3 replicates

fumigation chambers. This condensation indicated that moist air was reaching the blocks but signs of dryness appeared after 7 or 8 days. CFU declines in controls may reflect loss of viability due to drying during the exposure period, although declines did not always occur. For example, CFU's of *A. carbonica* growing on pine increased with time. Despite declines in some species, differences between control and chemically exposed samples were generally of a magnitude which permitted comparisons between the treatments.

Fumigations with carbon disulfide appeared to stimulate growth in most of the treatments between the first and fourth day of exposure (Fig. 2 through 5). Increases in CFU's exceeding 350 % with *P. placenta* on Douglas-fir and pine (Fig. 2b and 2c) were obtained.

The lowest level of carbon disulfide (500 ppm) had minimal effect on CFU's for all the species tested. As with the controls, CFU's for *G. saepiarium* and *H. resinae* (Fig. 3) exceeded 100% after ten days of fumigation. Many fungi produce thick-walled chlamydospores which are able to withstand long exposures under adverse environmental conditions or the presence of toxic substances (Zabel and Morrell, 1992). Observation of slide cultures under the microscope showed differences in the kind of spores produced by the fungi studied. *Antrodia carbonica* produced large amounts of thick-walled chlamidospores while none were observed for *I. lacteus* which was less tolerant to the treatments.

Differences in the number of CFU's between species reflect the ability to react to environmental changes,

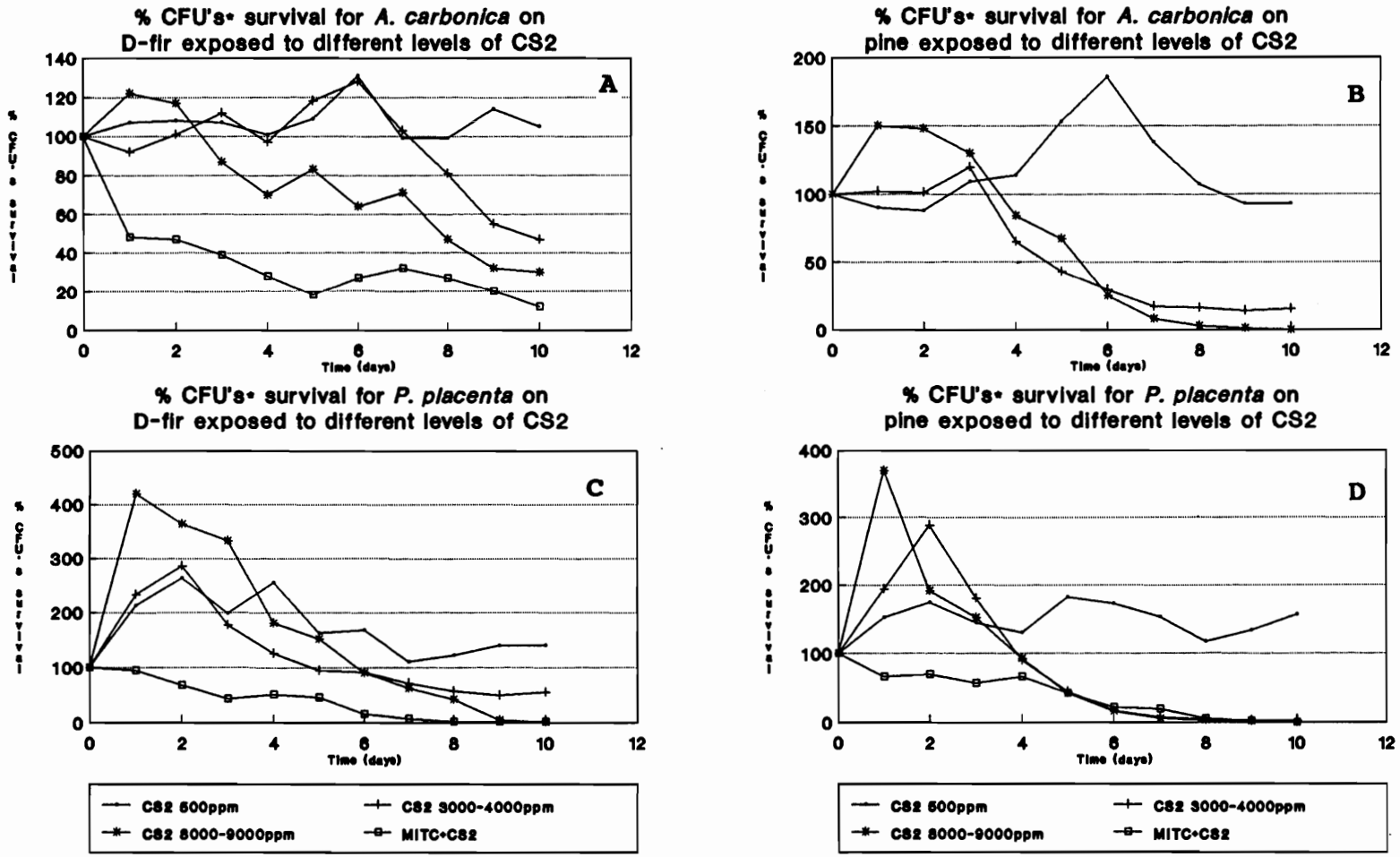


Fig.2 -Effect of exposure to 3 levels of CS_2 or a CS_2/MITC mixture on survival of *A. carbonica* and *P. placenta* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

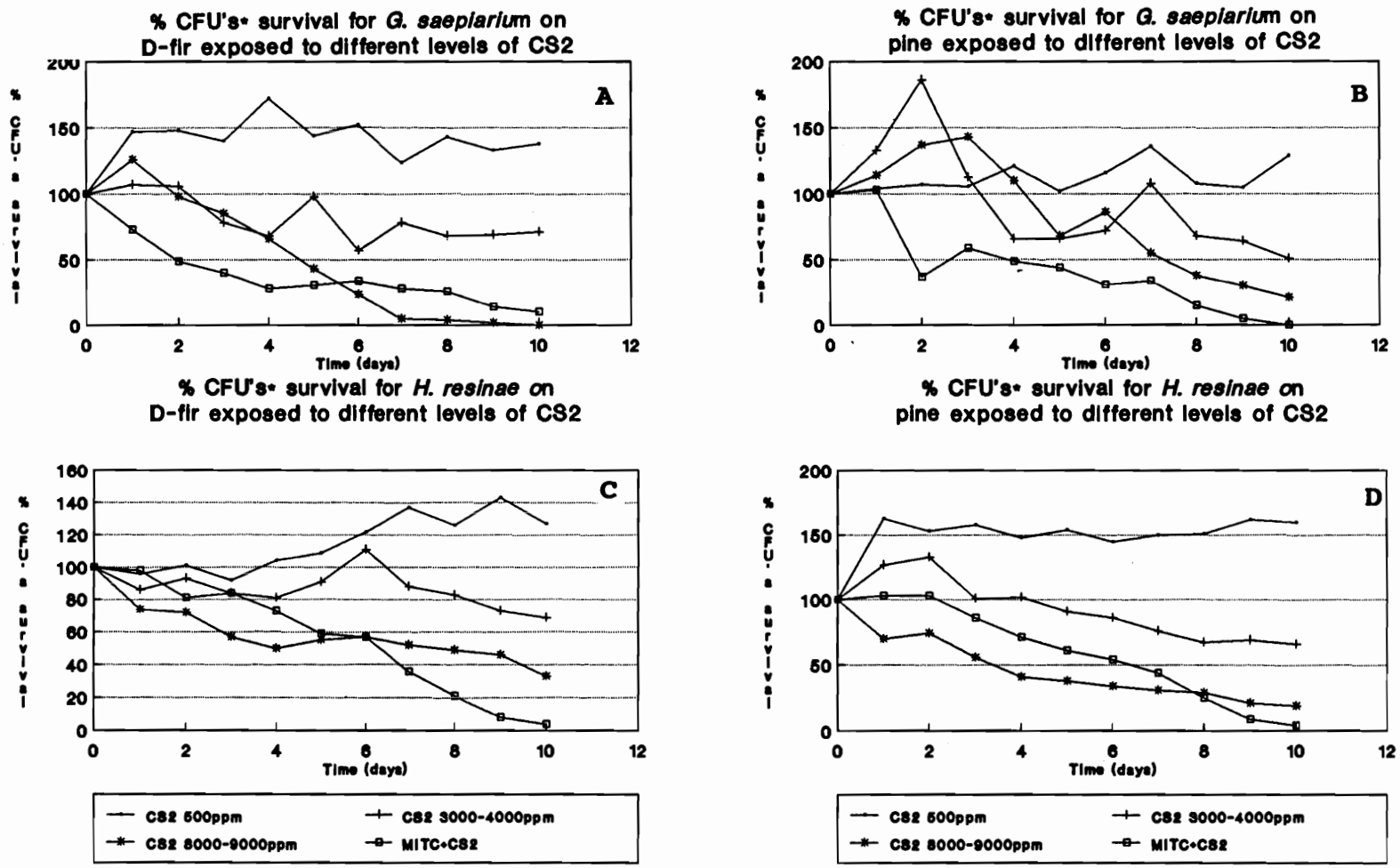
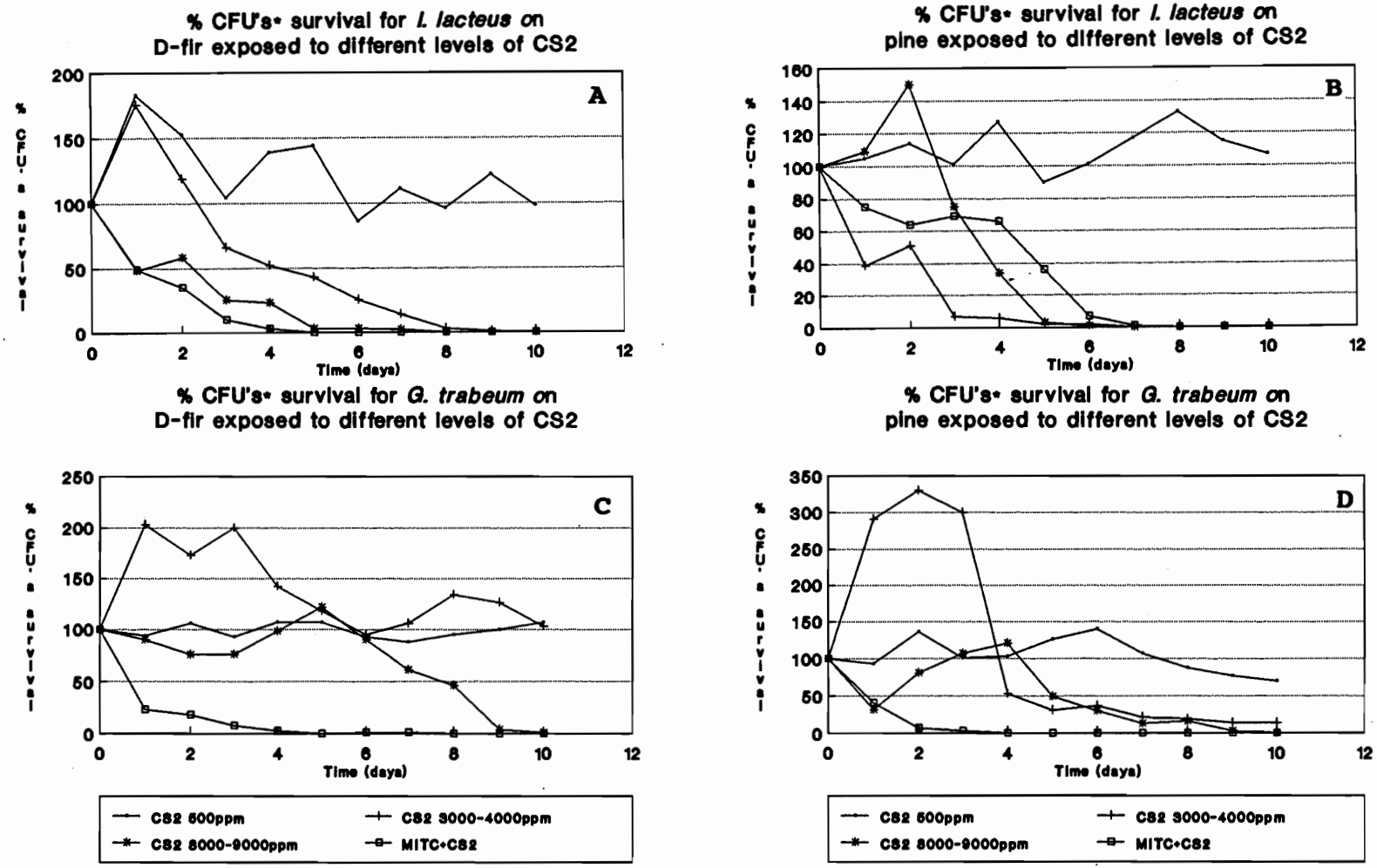


Fig.3 -Effect of exposure to 3 levels of CS_2 or a CS_2/MITC mixture on survival of *G. saepiarium* and *H. resinae* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.



* Colony Forming Units

Fig.4 -Effect of exposure to 3 levels of CS_2 or a CS_2/MITC mixture on survival of *I. lacteus* and *G. trabeum* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

especially with regard to amount and types of resistant structures produced. For example, *G. trabeum* was characterized by high CFU's at the beginning of fumigation, but these levels declined rapidly with time, suggesting this species was very susceptible to fumigation under the conditions of this experiment. Both *G. trabeum* and *G. saepiarium* produce large amounts of thin-walled arthrospores. These spores may be less resistant to fumigation than chlamydospores. *Trametes versicolor* was also stimulated by fumigation (Fig. 5), but the values obtained for this species were extremely variable.

Increasing carbon disulfide levels to 3000 to 4000 ppm produced declines in CFU's for virtually all of the fungi tested, but this level was only toxic to *I. lacteus* on Ponderosa pine (Fig. 4b). Several other fungi experienced declines in CFU's which exceeded the controls, but none succumbed to this fumigant level.

Exposure to 8000 to 9000 ppm of carbon disulfide resulted in decreased CFU's for all of the fungi tested, although several species survived a 10 day exposure to this chemical level. Initial stimulation of CFU's were again noted for *A. carbonica*, *P. placenta* (Fig. 2), or *G. saepiarium* (Fig. 3 a and b) on Douglas-fir or pine; and for *I. lacteus* and *T. versicolor* on pine. Very low levels of survival were noted with *P. placenta* on both pine or Douglas-fir, *A. carbonica* on pine, *T. versicolor* on pine or Douglas-fir and *G. saepiarium* on Douglas-fir upon prolonged exposure, suggesting that the fumigant might be effective upon longer exposures. Drying of wood in the fumigation system in longer exposures would, however, cause a corresponding decrease in CFU's as a result of desiccation,

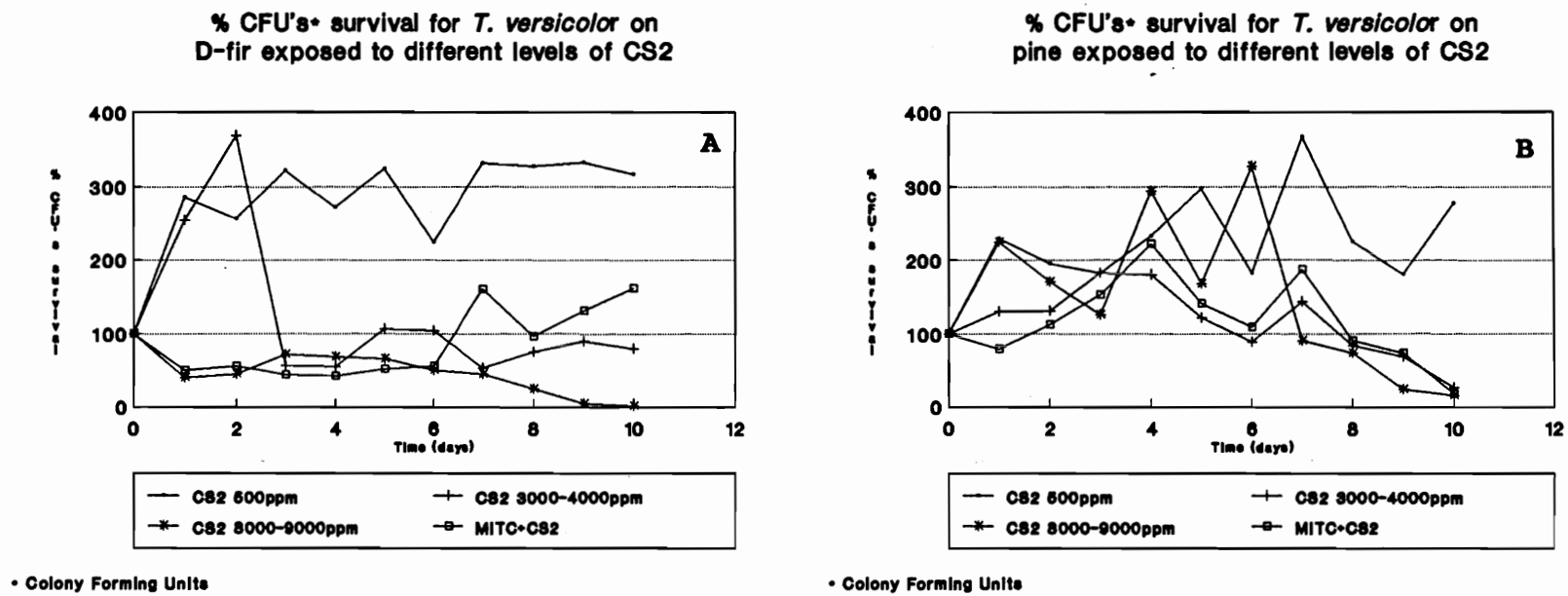


Fig.5 -Effect of exposure to 3 levels of CS_2 or a CS_2/MITC mixture on survival of *T. versicolor* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

obscuring potential fumigation effects. Several fungi, including *A. carbonica* on Douglas-fir (Fig. 2a), *H. resinae* on Douglas-fir or ponderosa pine (Fig. 3 c and d), and *G. saepiarium* on ponderosa pine (Fig. 3b) were relatively unaffected by exposure to 8000 - 9000 ppm carbon disulfide. *Antrodia carbonica* is an important colonizer of Douglas-fir heartwood and the survival of this species in the presence of carbon disulfide would be a major drawback if this fumigant were the only decomposition product of metham sodium (Graham and Corden, 1980; Eslyn, 1970). Similarly, *G. saepiarium* is an important colonizer of untreated pine, although studies suggest that this species is not an important colonizer of preservative treated southern pine (Zabel et al, 1980). The survival of *H. resinae* (Fig. 3 c and d) at the highest carbon disulfide level is not surprising in the light of the well known tolerance of this species to a variety of biocides. This fungus was occasionally isolated from Douglas-fir poles (Zabel et al., 1980), but was the most frequently isolated fungus from creosote-treated southern pine utility poles (Zabel et al., 1985). The possible effects of this fungus on residual fumigant levels in wood are unclear. *Hormoconis resinae* is capable of utilizing creosote as a sole carbon source but its ability to utilize carbon disulfide is unknown (Marsden, 1954; Kerner-Gang, 1978).

These results demonstrate that exposure of wood colonized by various decay and non-decay fungi to low levels of carbon disulfide reduces the numbers of CFU's in the wood but these levels were generally not lethal. Metham sodium is a relatively short lived treatment characterized by reinvasion of Douglas-fir poles by decay fungi starting only 5 to 7 years after treatment (Helsing

Table 5.- Effect of fumigation with MITC on ability to produce colony forming units (CFU's) for seven species of fungi on pine or Douglas-fir as a % of controls. [a/]

Time (days)	*A. carbonica* D-fir	*P. placenta* D-fir	*P. placenta* Pine	*I. lacteus* D-fir	*I. lacteus* Pine	*G. trabeum* D-fir	*G. trabeum* Pine	*G. saeplarium* D-fir	*G. saeplarium* Pine	*T. versicolor* D-fir	*T. versicolor* Pine	*H.resinae* D-fir	*H.resinae* Pine
	5 ppm MITC												
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1	95	34	329	146	127	124	14	270	146	139	44	113	110
2	99	41	159	121	89	93	27	170	103	112	78	93	90
3	82	79	80	111	53	39	28	93	14	100	81	74	87
4	83	64	76	53	57	26	25	29	6	85	127	47	65
5	73	45	73	47	47	25	24	21	5	99	190	39	46
6	83	84	57	23	38	14	12	13	3	145	417	29	28
7	82	27	58	11	22	6	6	13	3	157	415	22	26
8	84	28	60	11	7	1	3	5	2	147	301	20	14
9	70	22	63	4	1	0	1	1	2	143	534	11	9
10	64	23	44	3	2	0	1	0	1	205	346	6	8
	10 ppm MITC												
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1	118	82	93	120	61	84	126	193	138	100	211	117	98
2	146	92	163	37	90	119	330	123	72	82	88	117	74
3	118	113	199	46	55	93	19	41	18	65	16	39	54
4	110	155	111	24	19	19	22	21	7	60	51	33	34
5	71	60	60	30	17	6	11	4	2	62	22	21	33
6	56	68	52	7	3	3	5	2	1	76	29	17	21
7	78	40	52	1	2	0	1	1	0	32	12	12	6
8	111	24	39	1	0	0	0	0	0	59	12	5	5
9	72	7	2	0	0	0	0	0	0	16	2	6	3
10	48	1	0	0	0	0	0	0	0	5	0	4	3
	18 ppm MITC												
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1	75	25	108	59	48	216	103	52	34	157	334	86	74
2	66	19	29	25	45	122	68	25	25	124	197	42	40
3	34	22	20	15	4	3	17	7	2	99	72	10	21
4	34	9	36	3	4	1	11	0	0	40	47	13	13
5	18	3	4	1	0	0	0	0	0	10	21	6	8
6	4	1	6	0	0	0	0	0	0	2	1	7	3
7	2	0	0	0	0	0	0	0	0	1	0	6	3
8	1	0	0	0	0	0	0	0	0	0	0	4	1
9	0	0	0	0	0	0	0	0	0	0	0	2	0
10	0	0	0	0	0	0	0	0	0	0	0	1	0

[a/]Values represent the average of 3 replicates

et al. 1984; Zabel, et al., 1985; Zabel and Wang, 1988). The limited residual time of NaMDC in the wood may permit survival of fungal propagules in zones where diffusion of decomposition products is limited. These zones might include wet pockets, knots or other wood defects. If decomposition conditions shift heavily towards production of carbon disulfide, selected fungi may survive and later be able to germinate as conditions again become suitable for microbial growth.

4.2 Fungitoxicity of MITC

As expected, MITC had a more dramatic effect on the number of CFU's than carbon disulfide for all the species in the study (Table 5). As with carbon disulfide, an initial stimulus of CFU's was noted during the first two days of fumigation, especially for the 5 and 10 ppm MITC treatments. In some cases, this effect was also noted for the 18 ppm treatment (Fig. 6c, 8c and 8d).

The lowest concentration tested (5 ppm) produced declines in CFU's for most species except for *A. carbonica* on Douglas-fir (Fig. 9). High levels of CFU's were found with *P. placenta* on pine (Fig. 8b), and *T. versicolor* (Fig. 8 c and d) on both pine and Douglas-fir. *Trametes versicolor* showed a significant increase in CFU's after the fifth or sixth day of exposure during this fumigation. This effect may reflect delayed stimulation under low fumigant concentrations, but the reasons for this lag in effect are unclear. Little or no fungal survival was found with *I. lacteus* on pine or Douglas-fir (Fig. 6 a and b) and both *G. saepiarium* (Fig. 7 a and b) and *G. trabeum* on pine

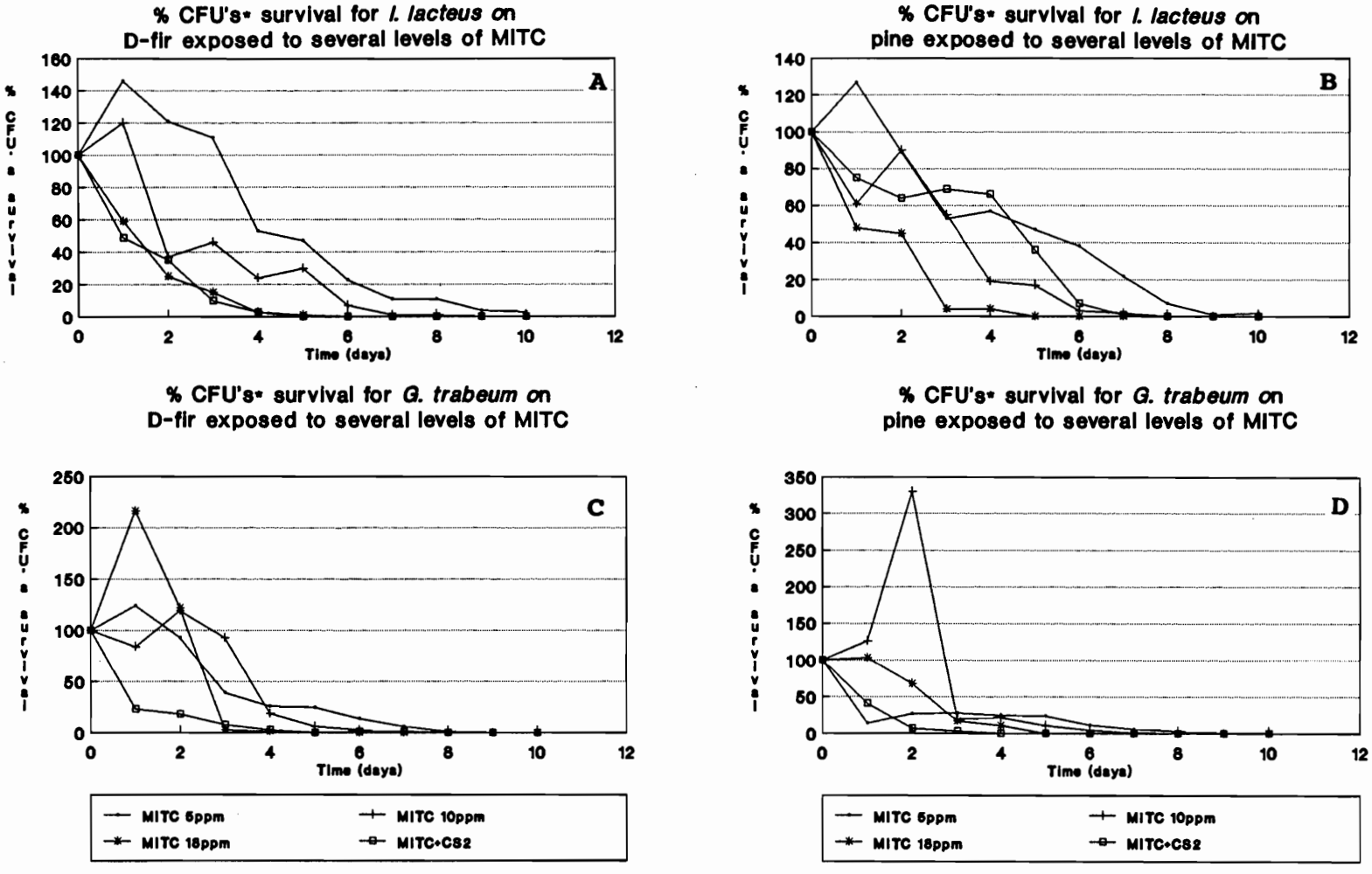


Fig.6 -Effect of exposure to 3 levels of MITC or a CS_2/MITC mixture on survival of *I. lacteus* and *G. trabeum* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

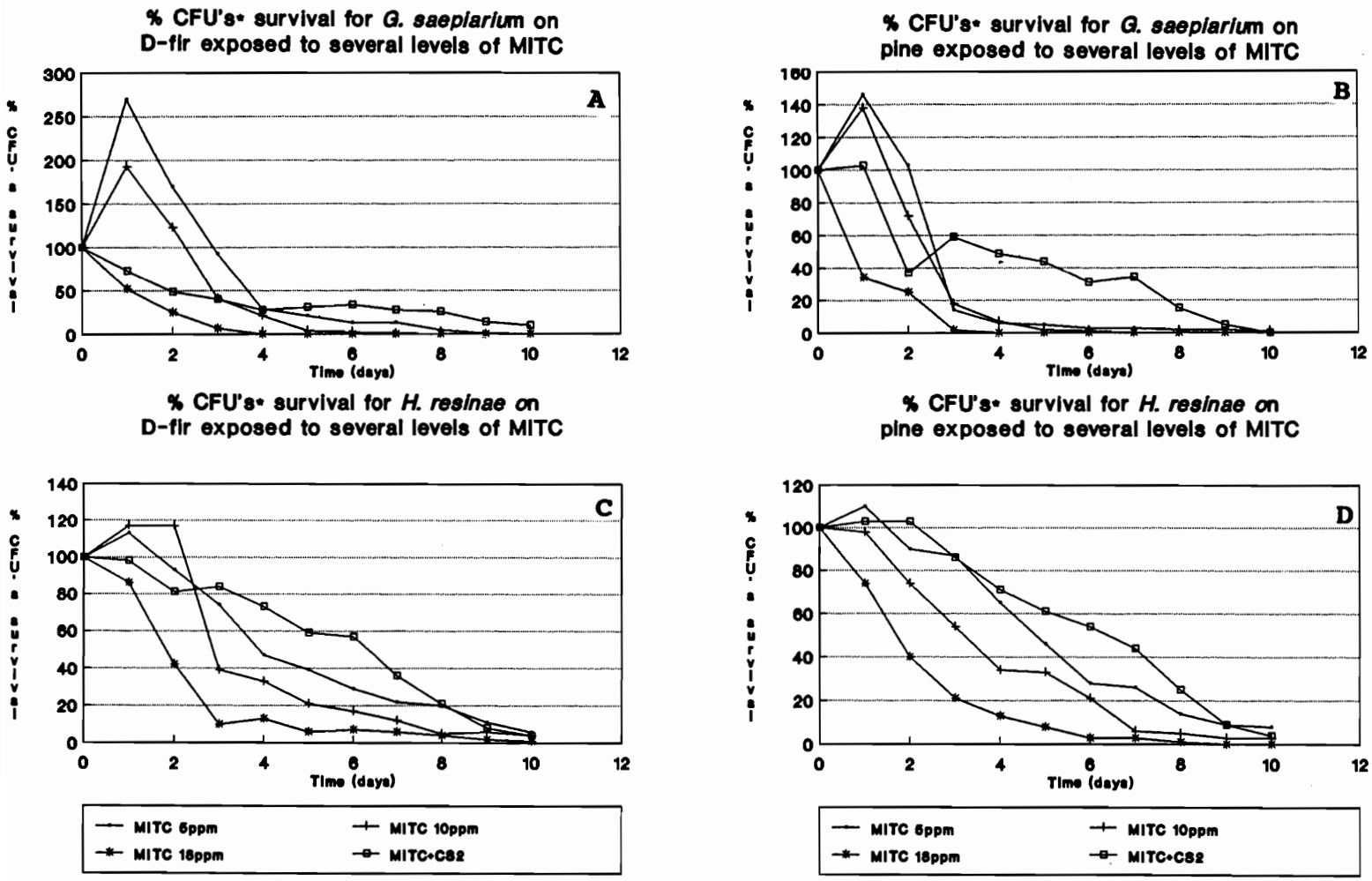


Fig.7 -Effect of exposure to 3 levels of MITC or a CS_2/MITC mixture on survival of *G. saepiarium* and *H. resinae* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

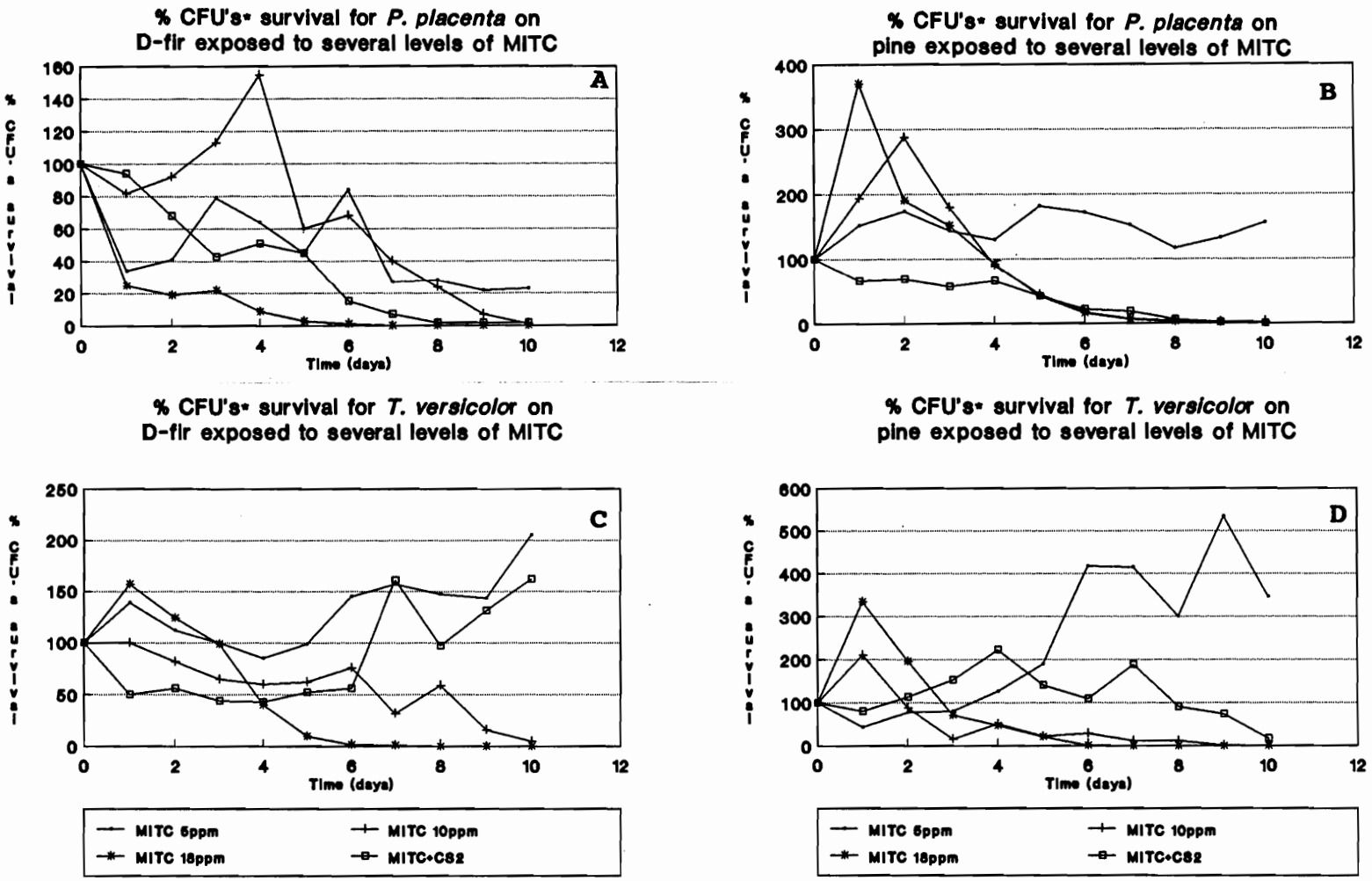


Fig.8 -Effect of exposure to 3 levels of MITC or a CS_2/MITC mixture on survival of *P. placenta* and *T. versicolor* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

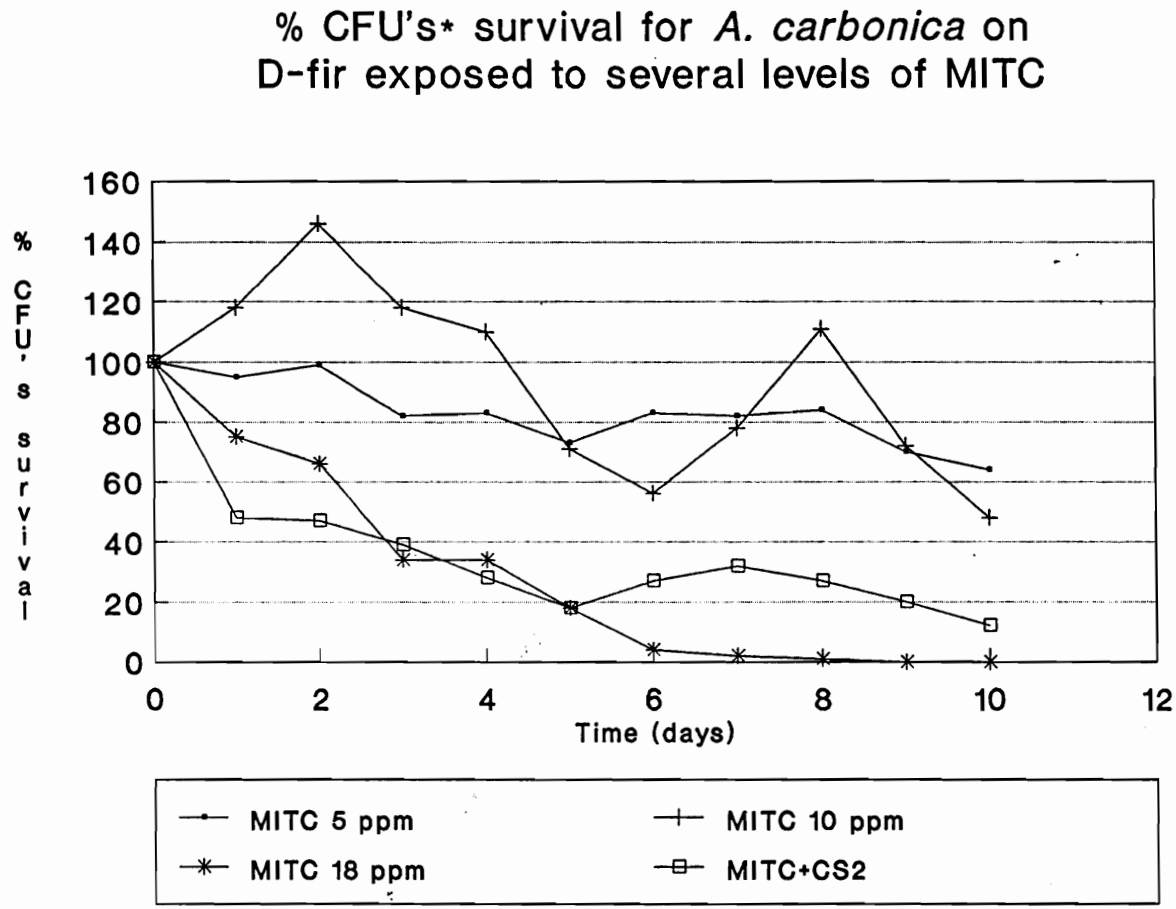


Fig.9 -Effect of exposure to 3 levels of MITC or a CS_2/MITC mixture on survival of *A. carbonica* on pine or Douglas-fir blocks as measured by number of colony forming units (CFU's) over a ten day exposure.

or Douglas-fir (Fig. 6 c and d). The presence of even limited surviving CFU's could be an important factor in wood "reinvasion" by fungi at points away from the fumigant application point were MITC levels might be expected to be low. The possibility of a surviving microflora in fumigated treated poles merits further study. Fungal recolonization of some wood species is often quite slow and the species present appear to differ from those found in some non-fumigant treated wood (Zabel et al, 1985; Giron and Morrell, 1989). Survival structures of some fungi may play a major role in this process.

Exposures to 10 ppm MITC produced a faster decline on CFU's for all species. *Irpex lacteus*, *G. trabeum* and *G. saepiarium* growing on both pine and Douglas-fir showed rapid declines in CFU's until the seventh to ninth day when these species succumbed (Fig. 6). Initial increases in CFU levels were also evident in most species during the first days of treatment as in carbon disulfide and 5 ppm MITC fumigations but the CFU's were much lower. Only *A. carbonica* (Fig. 9) and *H. resinae* (Fig. 7 c and d) survived a ten day exposure to 10 ppm MITC and even these species experienced marked CFU declines compared to the carbon disulfide and 5 ppm MITC treatments.

The highest rates of CFU decline were achieved with the 18 ppm MITC treatment. Only *A. carbonica* on Douglas-fir and *H. resinae* on pine or Douglas-fir produced viable colonies after 10 days of fumigation, although CFU levels were only 1 % of those found with the untreated controls.

4.3 Fungitoxicity of the mixture (MITC - CS_2)

Since both MITC and carbon disulfide are produced during NAMDC decomposition, these products may act synergistically to enhance fungal control. The initial results suggested that 5 ppm MITC and 3000-4000 ppm carbon disulfide produced CFU declines which while noticeable, were not lethal. These levels were subjected to further study.

Average CFU's for fungi exposed to a mixture of sublethal levels of carbon disulfide (3000 to 4000 ppm) and MITC (5 ppm) are shown on Table 6. Comparisons between the effect of MITC alone or with carbon disulfide on levels related to time of fumigation for each fungus are shown on Figures 2 through 9. Mixtures of sublethal dosages of carbon disulfide and MITC were generally more fungitoxic than MITC alone at either 5 or 10 ppm for *A. carbonica* on Douglas-fir, *P. placenta*, *I. lacteus*, *G. trabeum* and *T. versicolor*. The latter fungus exhibited increased CFU's for the 5 ppm fumigation on both pine and Douglas-fir (Fig. 8 c and d). This effect was absent with the mixture. Fumigant mixtures did not appear to have a noticeable effect on the number of CFU's of *H. resinae* and *G. saepiarium* in comparison to 5 ppm MITC but the mixture was more effective than carbon disulfide alone at 3000 to 4000 ppm. No CFU stimulus was noted for the mixture fumigation during first days of fumigation as with MITC and carbon disulfide alone (Fig. 7). It is important to note that sampling was performed every 24 hours. As a result, a short term stimulus earlier in the fumigation might be missed by our procedures.

Table 6.- Effect of fumigation with a mixture of 5 ppm MITC and 3000-4000 ppm CS_2 on the ability of seven species of fungi on pine or Douglas-fir to produce colony forming units (CFU's) as a % of controls. [a-/]

Time (days)	*A. carbonica*	*P. placenta*		*I. lacteus*		*G. trabeum*		*G. saepiarium*		*T. versicolor*		*H.resinae*	
	D-fir	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine
	5 ppm MITC												
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1	48	94	66	49	75	23	41	73	103	50	80	98	103
2	47	68	69	35	64	18	7	49	37	56	113	81	103
3	39	43	57	10	69	8	3	40	59	44	153	84	86
4	28	51	66	3	66	3	0	28	49	43	222	73	71
5	18	45	42	0	36	0	0	31	44	52	141	59	61
6	27	15	22	0	7	1	0	34	31	56	109	57	54
7	32	7	19	0	1	1	0	28	34	161	188	36	44
8	27	2	6	0	0	0	0	26	15	97	91	21	25
9	20	2	2	0	0	0	0	14	5	131	74	8	9
10	12	2	0	0	0	0	0	10	0	162	18	4	4

[a-/]Values represent the average of 3 replicates

Resistance and growth stimulation of fungal spores in the presence of sulfur containing fumigants has been observed in at least one previous study (Cobb, 1972). Cobb suggested that some fungi growing under sulfur-deficient regimes were more resistant and even stimulated when fumigated for two hours with MITC compared to the same species growing under sulfur rich conditions. Fumigant uptake and fungitoxicity in this earlier study were not always directly related. The lower the sulfur content of the spores, the higher the fumigant uptake by *Fusarium* spores and the greater the corresponding stimulation. Sulfur is necessary for fungal growth and reproduction (Lilly and Horace, 1951). The ability of fungi to oxidize sulfur *in vitro* has long been recognized (Waksman, 1918; Armstrong, 1921). Sulfur-containing compounds in fungal cells include enzymes and other proteins, free amino acids such as cysteine and methionine, and some vitamins (thiamine, biotin and coenzyme A) (Lilly, 1965; Slaughter, 1988). In addition to major sulfur compounds, fungi also produce a limited range of volatile sulfur compounds usually at low concentrations which appear to represent a more or less accidental loss of sulfur from the biological system. In many cases, these compounds do not seem to have any function in the intermediary metabolism of the fungus (Slaughter, 1988). Sulfur content in wood is very low, generally less than 0.1% for species like Douglas-fir and pine (Mingle and Boubel, 1968). It is possible that some fungi can metabolize MITC or CS_2 in order to satisfy sulfur needs. These effects may help to explain the initial stimulation in CFU's observed in most of the species fumigated with 5 and 10 ppm MITC, although more refined studies of sulfur balance in fumigated fungi would be required to confirm this effect.

4.4 Concentration time estimates.

Logarithm survival was related to total fumigant concentration (Concentration x Time) to which the blocks were exposed during fumigation with sublethal dosages. Figures 10 to 16 show the CxT curves for the effect of sublethal dosages of each chemical compared with that of the mixture of both gases. Table 7 presents the CxT values necessary to kill 90% (CT_{90}) of the CFU's for each fungus in each treatment. For most species, the fumigant mixture was more effective than either carbon disulfide or MITC alone. For example CT values for the mixture against *A. carbonica* on Douglas-fir were 5 times lower than MITC alone and over 3 times lower than CS_2 alone. For *P. placenta* on Douglas-fir CT_{90} for the mixture were nearly 3 times lower than MITC or CS_2 alone. In some instances, however, CT_{90} values for the mixture were higher than those for the individual components. CT_{90} values for the mixture against *I. lacteus* on pine were 1.5 times larger than CS_2 alone, that against *P. placenta* was equivalent to carbon disulfide and the CT_{90} for the mixture against *H. resinae* on pine or Douglas-fir was similar to that found for MITC. Finally CT_{90} for the mixture against *G. saepiarium* on Douglas-fir or pine were higher than CT_{90} for MITC.

An additional finding with the CT_{90} values was a tendency toward higher CT_{90} values for Douglas-fir at the 3000-4000 ppm carbon disulfide treatment. Douglas-fir heartwood is less permeable than pine sapwood (Panshin and DeZeeuw, 1980), however, this effect was not evident at higher CS_2 levels (8000-9000), MITC alone or the CS_2/MITC mixture.

Table7.- Concentration x Time (CT) values necessary to kill 90% of the propagules for seven species of fungi exposed to carbon disulfide, MITC or a CS_2/MITC mixture. Values in paranthesis represent the number of days necessary to reach 90% kill.

CT_{90}values ($x10^6$)								
		CS2		MITC			MIXTURE	
Fungus	Wood Species	3000-4000 ppm	8000-900 ppm	5 ppm	10 ppm	18ppm	CS2	MITC
A. carbonica	Douglas-fir	3172.18 (42)	3496.59 (19)	6.72 (62)	7.47 (35)	1.99 (5)	941.81	1.35 (2.5)
P. placenta	Douglas-fir	1544.08 (20)	1722.21 (9)	1.95 (18)	1.99 (9)	1.3(3.3)	496.26	0.71 (6.6)
	Pine	551.29 (7)	1192.71 (6.5)	2.21 (20.5)	1.81(8.4)	1.67(4.3)	525.97	0.75 (7)
I. lacteus	Douglas-fir	489.423 (6.5)	779.69 (4)	0.83 (7.7)	1.10 (5)	1.16 (3)	208.07	0.33 (3)
	Pine	253.19 (3)	798.18 (4)	7.85 (72)	1.05 (5)	1.12 (3)	391.24	0.56 (5)
G. trabeum	Douglas-fir	5337.48 (70)	1769.55 (9.7)	0.61 (5.6)	1.01 (5)	1.17 (3)	211.11	0.30 (3)
	Pine	764.19 (10)	1374.04 (7.5)	0.57 (5)	1.06 (5)	1.31(3.4)	167.41	0.07 (2)
G. saepiarium	Douglas-fir	3725.8 (49)	1237.33 (7)	0.69 (6)	1.03 (5)	0.96(2.5)	841.99	4.20 (11)
	Pine	2257.54 (30)	2916.02 (16)	0.54 (5)	0.83 (4)	0.82 (2)	570.64	0.82 (7.5)
T. versicolor	Douglas-fir	2312.71 (30)	1669.89 (9)	-4.38	2.60 (12)	1.93 (5)	-1155.80	-1.64
	Pine	2010.45 (27)	3140.57 (17)	-8.15	1.44(6.7)	1.93 (5)	2299.13	3.28 (30)
H. resinae	Douglas-fir	7484.86 (100)	4872.45 (26)	1.03 (9.5)	1.60(7.4)	1.98 (5)	739.16	1.06 (10)
	Pine	2818.80 (37)	2538.99 (14	1.03 (9.5)	1.49 (7)	1.83(4.7)	759.71	1.09 (10)

Douglas-fir permeability to MITC is largely influenced by moisture content in wood (Zahora, 1987). Very low MITC concentrations, which may not be toxic to inactive decay fungi in dry wood, become fungitoxic in wet wood. Increased susceptibility of *A. carbonica* to MITC in wet wood may be important in determining long-term wood protection, since fungal growth and active decay will only occur in wood above fiber saturation point. In the present study, moisture content in wood was maintain above fiber saturation point which may help to explain the fungitoxicity of the fumigants despite the low MITC concentrations employed.

The results from the present study suggest a synergism interaction between MITC and carbon disulfide that may enhance metham sodium fungitoxicity. This type of synergism, if present between other metham sodium decomposition products, may help to explain the relatively strong performance of this fumigant in wood. Recent studies have suggested that metham sodium may be improved by the incorporation of additives which shift decomposition towards MITC production (Morrell, in press; Lebow and Morrell, 1993). These practices may enhance MITC production, but their impact on the rate of elimination of fungal infestation may be diminished by the loss of synergistic decomposition products such as CS_2. These possibilities suggest that further studies of the interaction between MITC and other volatile decomposition products are warranted before changes in metham sodium formulations are considered.

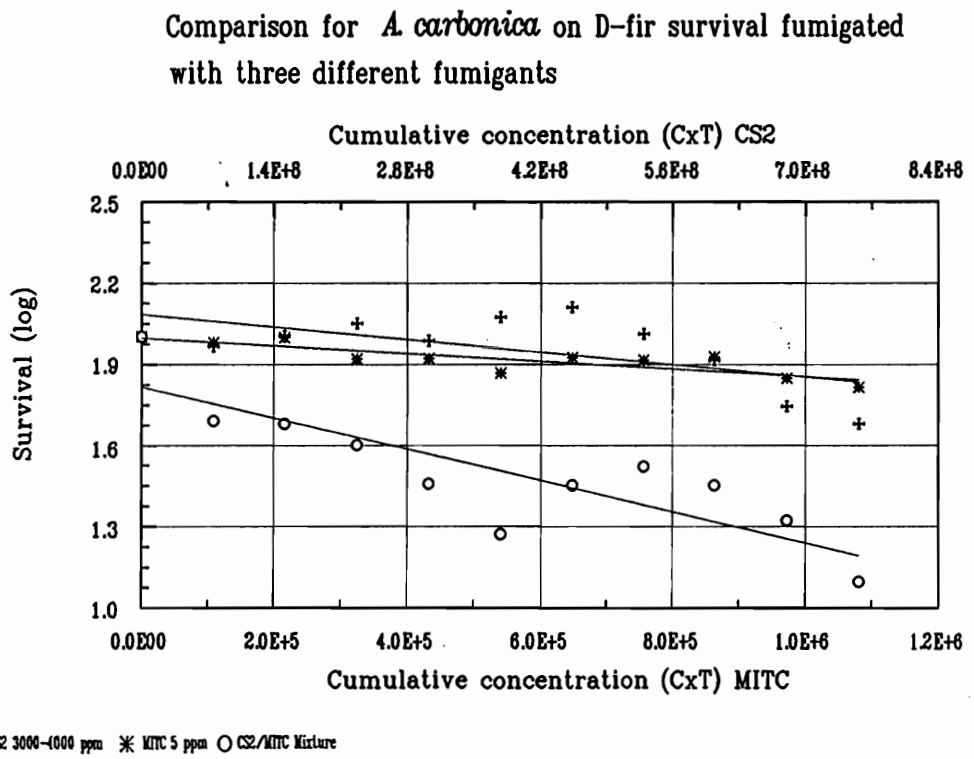
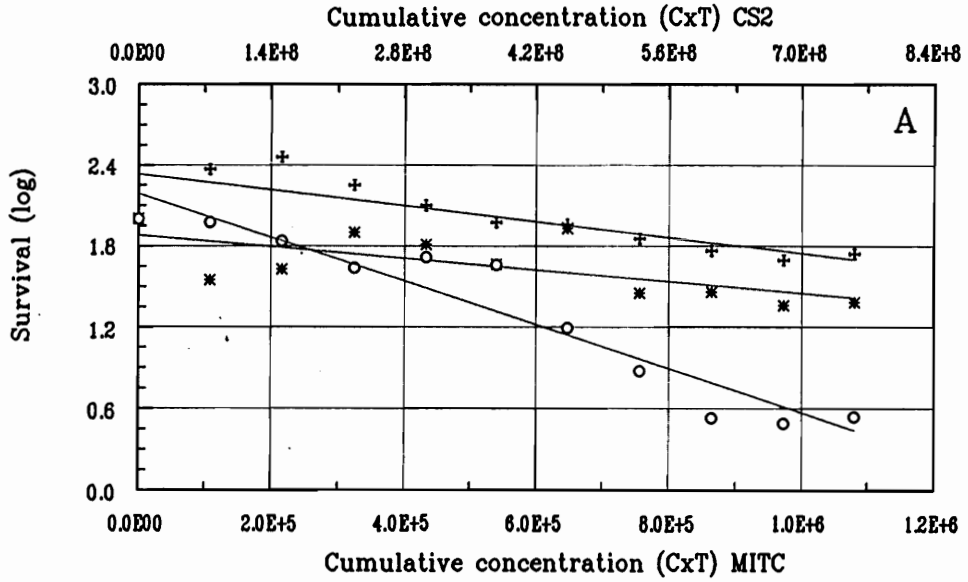


Fig.10 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *A. carbonica*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).



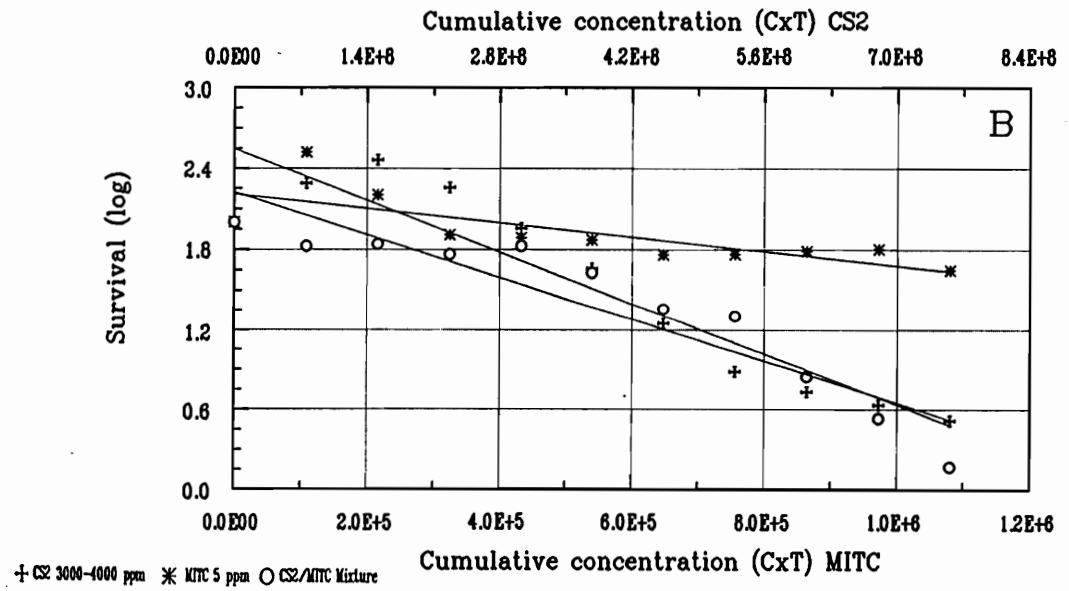


Fig.11 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *P. placenta*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

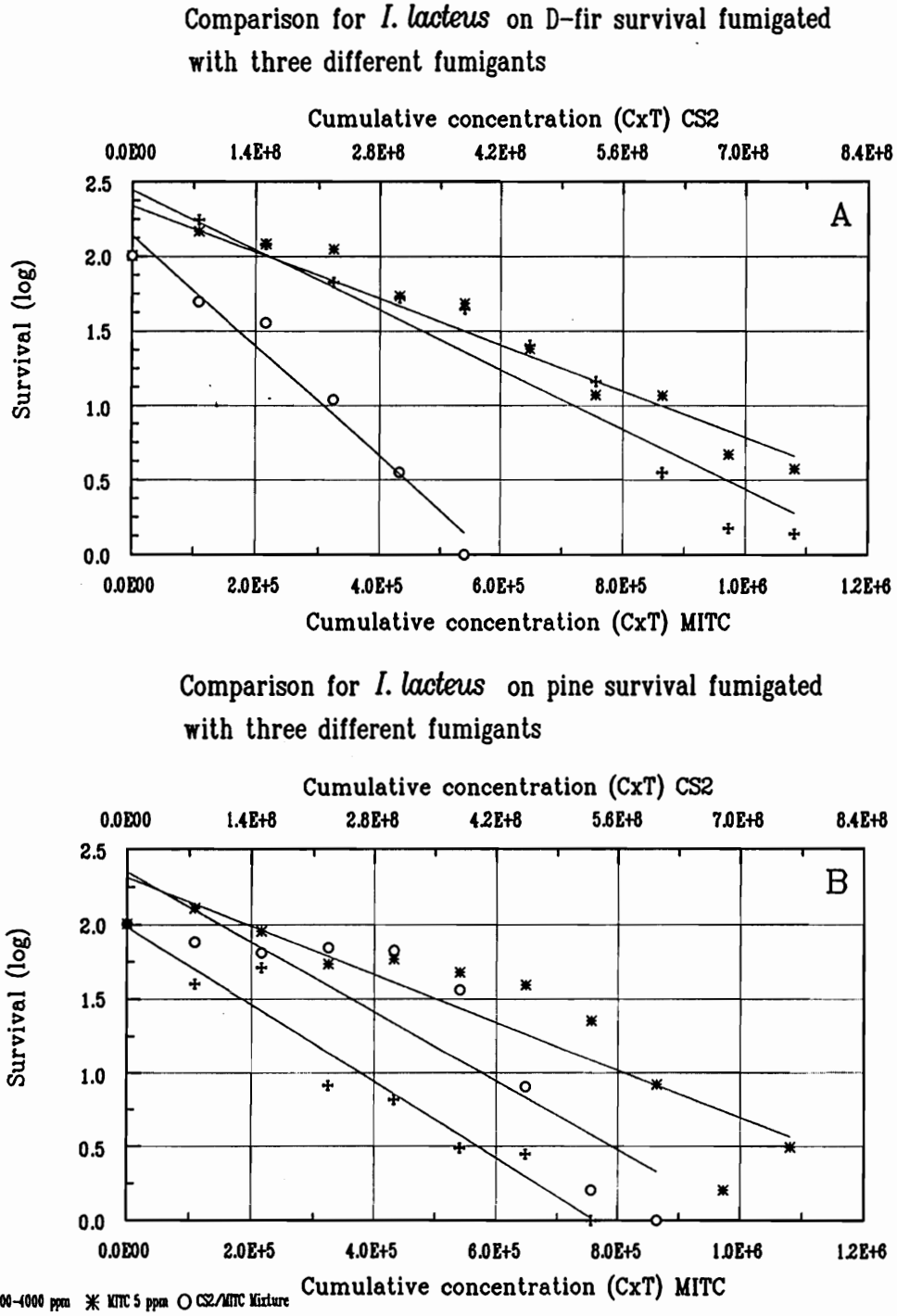


Fig.12 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *I. lacteus*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

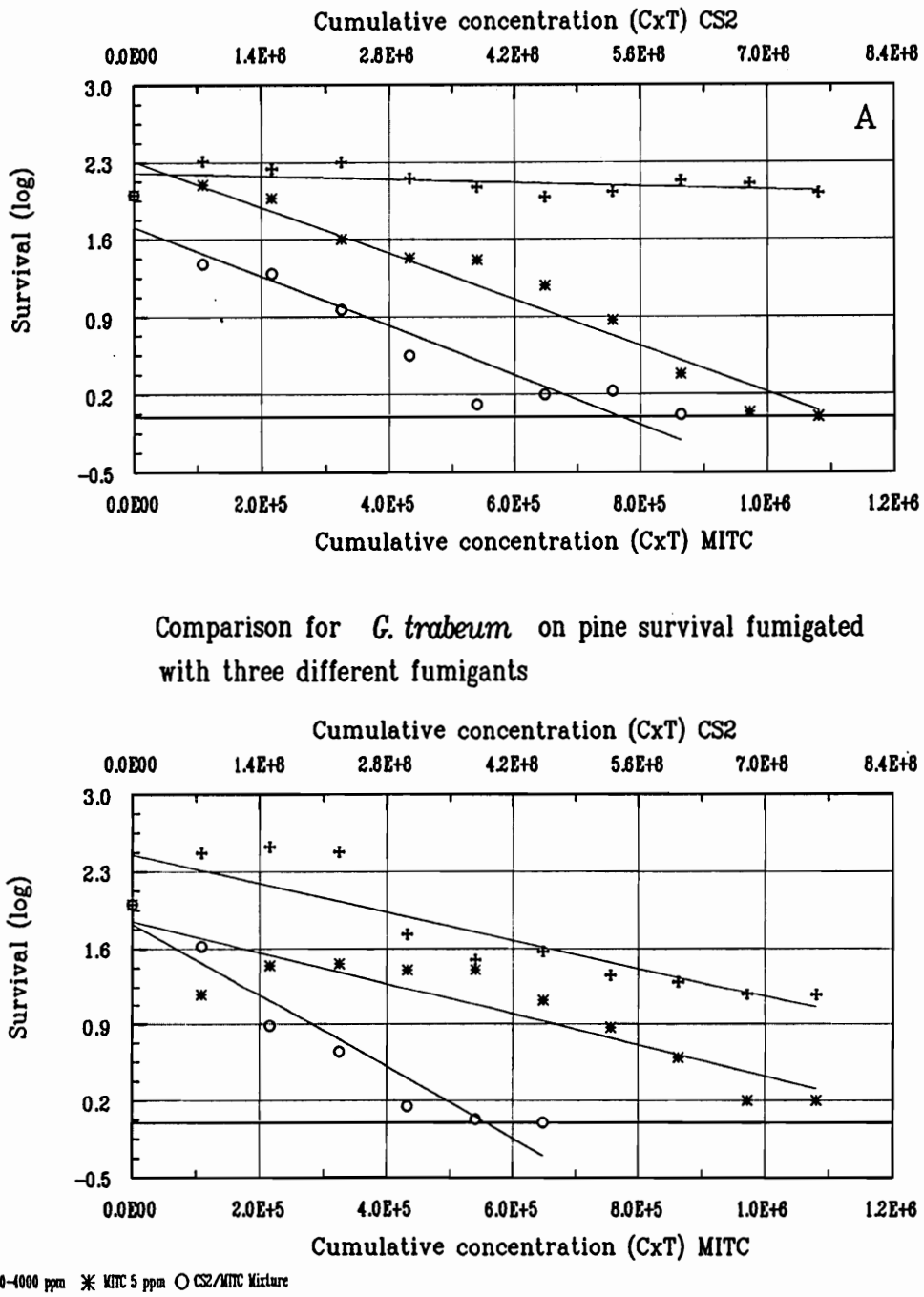
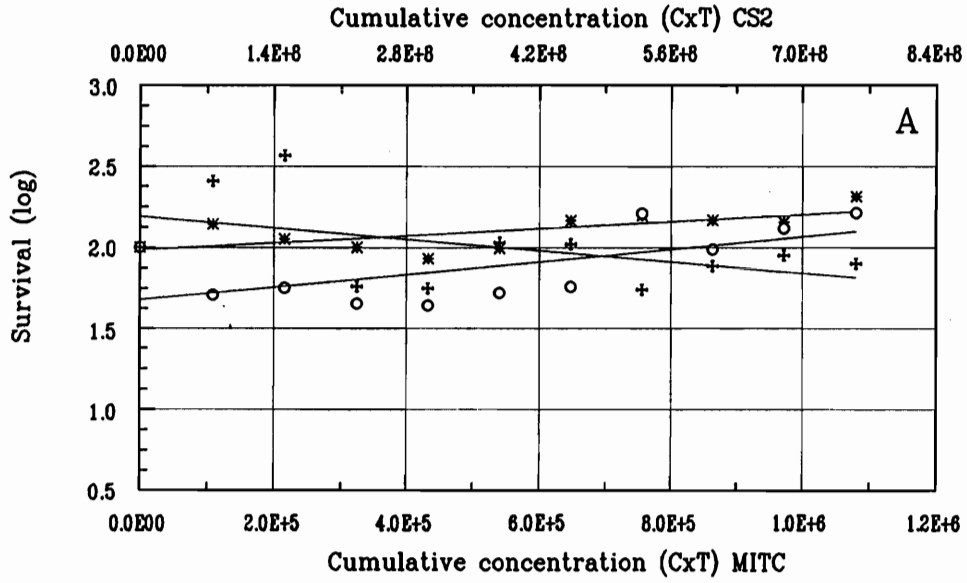


Fig.13 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *G. trabeum*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

Comparison for *T. versicolor* on D-fir survival fumigated with three different fumigants



Comparison for *T. versicolor* on pine survival fumigated with three different fumigants

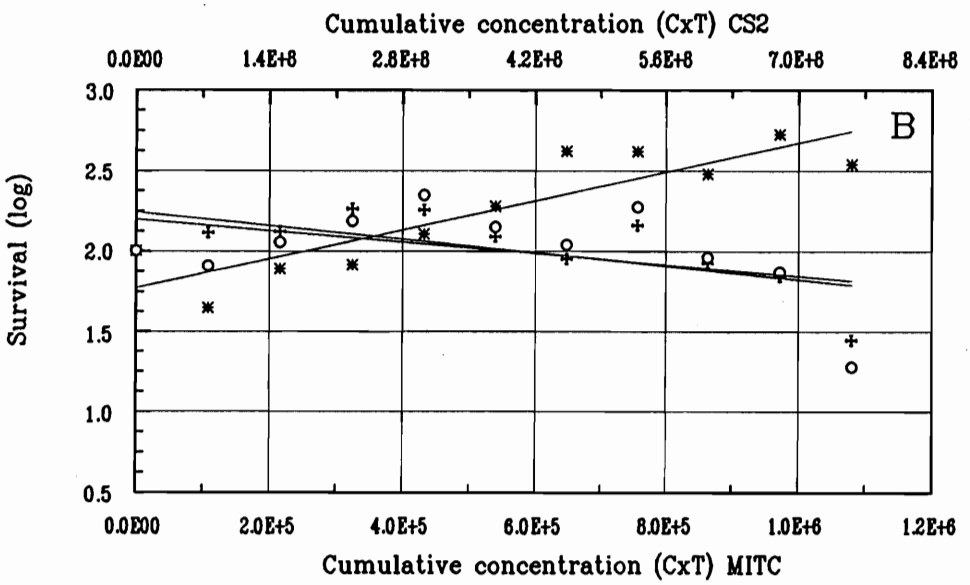


Fig.14 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *T. versicolor*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

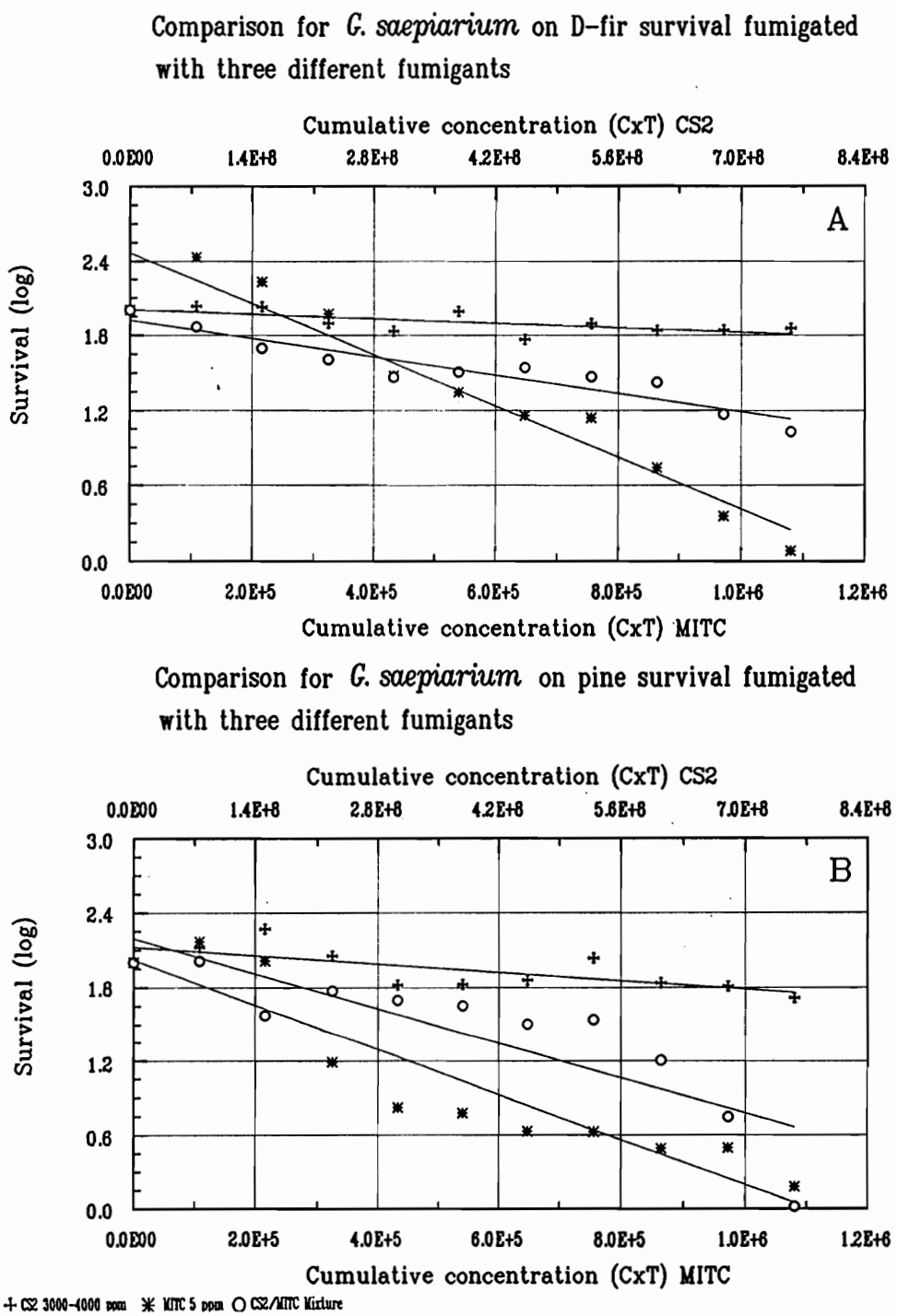
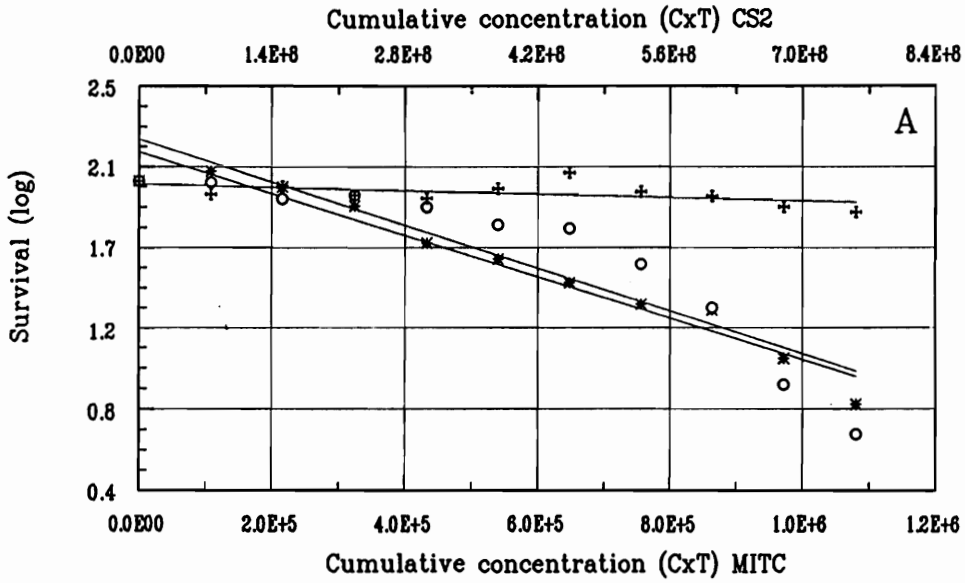


Fig.15 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *G. saepiarium*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

Comparison for *H. resinae* on D-fir survival fumigated with three different fumigants



Comparison for *H. resinae* on pine survival fumigated with three different fumigants

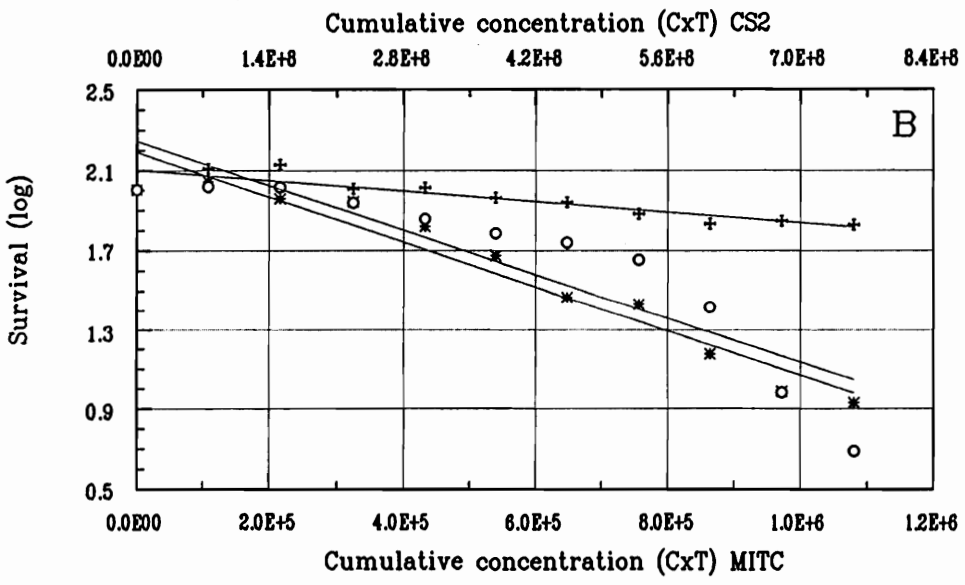


Fig.16 -Effect of sublethal dosages of CS_2 and MITC along with CS_2/MITC mixture on survival of *H. resinae*. The Y axis represents the logarithm of survival and both X axis represent the product of concentration multiplied by time for MITC (lower axis) and carbon disulfide (upper axis).

V
Conclusions

The results illustrate the wide degree of variation in response of fungi to fumigation as well as the difficulty of completely eliminating all fungi from wood using this control method. The results, however, must be viewed with some caution, since the isolation procedures employed to measure colony forming units may have inadvertently influenced survival of one species over another. Despite these considerations, the results can be readily used to test the relative effects of a chemical on a given species.

Although considered to be less important in the use of gas phase treatments, the wood species in which a fungus was established appeared to have some effect on survival of some species. This effect may reflect different diffusion rates or sorption/desorption values for the various wood species, or a different fungal growth pattern between the species. These results suggest that fumigant studies using different wood species should not be broadly applied.

The presence of growth stimulus at the start of fumigation was an interesting phenomenon which may reflect fungal adaptation to low sulfur environments. The significance of this effect in practice is difficult to ascertain. Near the site of treatment, fungi which are initially stimulated, will ultimately succumb to continued fumigation. Colonies further from the treatment site may receive sublethal dosages of fumigant and be little affected by such treatments. This possibility, particularly above the site of fumigation merits further study.

The interactive effects of MITC and CS_2 suggest that the fungicidal action of metham sodium in wood is far more complex than previous thought. While synergistic activity among biocides is well documented, the possibility that the various metham sodium decomposition products can interact to enhance activity helps to explain the higher activity of this compound in wood. The results also suggest that interactions may occur among the other decomposition products. Metham sodium decomposes to over 14 possible compounds. Of these, only MITC and CS_2 have been studied in wood. Further studies using other volatile decomposition products such as methylamine and hydrogen sulfide may provide important clues concerning the factors which maximize fungitoxicity of this fumigant in wood and could be used to enhance decomposition to produce the compounds which most likely to effect fungal control.

VI

Literature Cited

Armstrong, G. M. 1921. Studies in the physiology of the fungi-sulfur nutrition, the use of thiosulphate as influenced by hydrogen ion concentration. Annals of the Missouri Botanical Garden 8:237-248.

Bessey, E.A. 1950. Morphology and taxonomy of fungi. Hafner Press. New York, N.Y. 791 pp.

Blis, D. E. 1951. The destruction of *Armillaria mellea* in citrus soils. Phytopathology 41:665-683.

Briscoe, C.B. and F.R. Strickland. 1956. Vapam shows promise as forest nursery herbicide. Tree Planters Notes. 26:3-4. From Forestry Abstracts 18(2):198.

Cobb, W.T. 1972. The influence of sulfur and carbohydrate nutricion of *Fusarium oxysporum* F. sp. *Lycopersici* on resistance to methylisothiocyanate. PhD. Dissertation. Oregon State University. Corvallis, OR. 59 pp.

Cooper P.A., R.D. Graham and R.T. Lin. 1974. Factors influencin the movement of chloropicrin vapor in wood to control decay. Wood and Fiber 6(1):81-90.

Corden, M.E.; J.J. Morrell; M.A. Newbill and R.D. Graham. 1988. Fumigant protection of untreated Douglas-fir posts. Holzforschung 42(2):127-130.

Elson, J.E. 1966. Fungitoxicity of sodium N-Methy-dithiocarbamate (Vapam) and its decomposition products. M.A. Thesis. Oregon State University. Corvallis, OR. 35 p.

Eslyn, W.E. 1970. Utility pole decay. II Basidiomycetes associated with decay in poles. Wood Science and Technology 4:97-103.

Fengel, D. and G. Wegener. 1984. Wood. Chemistry ultrastructure reactions. W. de Gruyter. Berlin. 613p.

Filip, G. M. and L. F. Roth. 1977. Stump injections with soil fumigants to erradicate *Armillaria mellea* from young-growth ponderosa pine killed by root rot. Canadian Journal of Forestry Research 7:226-231.

Foster, A.A. 1956, Fumigation of forest nurseries in the southeast (USA) for control of weed and root rot. Tree Plant Notes.No. 26:1-2.

Garret, S. D. 1957. Effect of a soil microflora selected by carbon disulfide fumigation on survival of *Armillaria mellea* in woody host tissues. Canadian Journal of Microbiology 3:135-149.

Giron, M.Y., and J.J. Morrell. 1989. Fungi colonizing preservative treated Douglas-fir poles after remedial treatment with fumigants. Canadian Journal of Microbiology. 35:283-288.

Goodell B.S. and R.D. Graham. 1983. A survey of methods use to detect and control fungal decay of wood poles in service. International Journal of Wood Preservation 3(2):61-63.

Goodell B.S., R.D. Graham and R.L. Krahmer. 1980. Chloropicrin movement and fungitoxicity in decaying southern pine laminated timber. Forest Products Journal 30(7):39-43.

Goodell B.S., R.L. Krahmer, and R.D. Graham. 1985. Residue retention and fungal invasion of chloropicrin- treated Douglas-fir. Forest Products Journal 35(2)45-49.

Goring, C. A. 1967. Physical aspects of soil in relation to the action of soil fungicides. Annual Review of Phytopathology 5:285-318.

Graham, R.D. 1961. Sink-float test to determine treatability of Douglas-fir. Forest Products Journal 14(11):516.

Graham, R. D. 1973. History of wood preservation. In: Wood deterioration and its prevention by preservative treatments (D.D Nicholas, Ed.) Syracuse University Press, Syracuse, N.Y. 1-30 pp.

Graham, R. D. 1983. Improving the performance of wood poles. Proceedings American Wood Preservers Association 79:222-228.

Graham, R. D. and M.E. Corden. 1980. Controlling biological deterioration of wood with volatile chemicals. Electric Power Research Institute. Report EL1480. Final Report. Oregon State University.

Hand O.F., P.A. Lindgren and A.F. Wetsch. 1970. The control of fungal decay and insects in transmission poles by gas phase treatment. Bonneville Power Administration. Branch of Laboratories. Vancouver, Washington. 28pp.

Helsing, G.G.; J.J. Morrell and R.D. Graham. 1984. Evaluation of fumigants for control of internal decay in pressure-treated Douglas-fir poles and piles. Holzforschung 38(5):277-280.

Johnson, M.O.and G.H. Godfrey. 1932. Chloropicrin for nematode Control. Industrial Engineering Chemistry 24:311-313.

Jones, T.W. 1963. Fumigation may end oak embargoes. Forest Products Journal 13(12):564.

Kerner-Gang, W. 1976. Effect of microorganisms on creosote. International Symposium. Berlin-Dahlem. 1975. Material und Holz. Beiheheft. 3:319-330.

Kuntz, J.E. and C.R. Drake. 1960. Control of the local spread of oak wilt with soil fumigants. Forestry Research Note. Wisconsin College of Agriculture. No. 68. pp. 6. From Forestry Abstracts. 22(4):599.

Lebow, S.T. and J.J. Morrell. 1993. Methylisothiocyanate fumigant content of Douglas-fir heartwood at various moisture levels after treatment with solid sodium n-methyldithiocarbamate. Wood and Fiber Science 25(1):87-90.

Lilly, V. G. 1965. Chemical constituents of the fungal cell, 1. Elemental constituents and their role. In: The fungi: An advanced treatise. Vol 1. (Ed. by G. C. Ainsworth and Alfred S. Sussman). New York Academic Press. New York. p 163-175.

Lilly, V. G. and L. B. Horace. 1951. Physiology of the fungi. McGraw-Hill Book Co. New York.

Marsden, D. H. 1954. Studies on the creosote fungus *Hormodendrum resinae*. Mycologia 46:181-183.

Matheron, M.E. and J.C. Matejka. 1988. In vitro activity of sodium tetrathiocarbonate on sporulation and growth of six *Phytophthora* species. Phytopathology 78(9):1234-1237.

McCallan, S.E. 1967. History of fungicides. In: Fungicides: An advance treatise. (Dewayne C. Torgeson Ed). Academic Press. New York. N.Y. p 1-29.

Micklewright, J.T. 1990. Wood preservation statistics, 1988. A report to the wood preserving industry in the United States. Proceedings American Wood Preservers Association 86:1258-272.

Miller, J.D. 1961. Treatability of Douglas-fir from western United States. Proceedings American Wood Preservers Association 57:218-233.

Miller, D.B. and J.J. Morrell. 1989. Stability and methylisothiocyanate production of 12 potential solid fumigants for controlling wood decay. Holzforschung 43(3):191-194.

Miller, D.B. and J.J. Morrell. 1990. Interactions between sodium N-methyldithiocarbamate and Douglas-fir heartwood. Wood and Fiber Science 22(2):135-141.

Miller, L.P.; S.E. McCallan and R.M. Weed. 1953. Quantitative studies on the role of hydrogen sulfide formation in the toxic action of sulfur to fungus spores. Contribution of the Boyce Thompson Institute 17(2):151-171.

Mingle, G. J. and R. W. Boubel. 1968. Proximate fuel analysis of some western wood and bark. Wood Science 1(1):29-36.

Molin, N.; J. Tear. 1956. Chemical weed control in nurseries. Medd. SkosforsknInst. Stockh. 46(6). From Forestry Abstracts 18(1):198. Abs. 1530.

Morrell, J.J. Effect of wood species, temperature and moisture content on decomposition of metham sodium to methylisothiocyanate. Wood and Fiber Science (in press).

Morrell. 1989. The use of fumigants for controlling decay of wood: a review of their efficacy and safety. International Research Group on Wood Preservation Document No. IRG/WP/3525. Stockholm, Sweden. 17p.

Morrell, J.J., and M.E. Corden. 1986. Controlling wood deterioration with fumigants: A review. Forest Products Journal 36(10):26-34.

Morrell J.J. and S.T. Lebow. 1989. Volatile emissions from Douglas-fir heartwood treated with Vapam or methylisothiocyanate. Forest Products Journal 39(2):41-44.

Morrell, J.J.; C.M. Sexton and S.T. Lebow. 1988. The effect of pH on decomposition of Mylone (Dazomet) and tridipam to fungitoxic methylisothiocyanate in wood. Wood and Fiber Science 20(4):422-430.

Munnecke, D. E.; M. J. Kolbezen and W. D. Wilbur. 1973. Effect of methyl bromide or carbon disulfide on *Armillaria* and *Trichoderma* growing on agar medium and relation to survival of *Armillaria* in soil following fumigation. Phytopathology 63:1352-1357.

Newhall, A.G. 1955. Disinfectation of soil by heat, flooding and fumigation. Botanical Review 21:189-250.

Panshin, A.J. and C. DeZeeuw, 1980. Textbook of wood technology. McGraw Hill. New York. N.Y. 705 pp.

Parrish, G.K. 1958. Soil fumigants and their use. Plant Desease Reporter 42(2):273-278.

Patridge, A.D. 1961. The oak wilt fungus in wood. Forest Products Journal 11(1):12-14.

Ricard J.L., T.E. See and W.B. Bollen. 1968. Control of incipient decay with gases in Douglas-fir poles. Forest Products Journal 18(4):45-51.

Scheffer, T.C. 1973. Fumigants can stop internal decay of wood products. Forest Products Journal 23(2):168-173.

Scheffer, T.C., and R.D. Graham. 1975. Bioassay appraisal of vapam and chloropicrin fumigant-treating for controlling internal decay of Douglas-fir poles. Forest Products Journal 25(6):50-56.

Sexton, C. M.; P. G. Forsyth and J. J. Morrell. Preparation of basidiomycete colonized wood using a vermiculite burial method. Phytopathology (In Press).

Smelt, J.H. and M. Leistra. 1974. Conversion of metham-sodium to methylisothiocyanate in soil. Pesticide Science 5:401-407.

Smith D.M. and R. Cockroft. 1967a. The remedial treatment of telephone and electric transmission poles. Part 1. Treatment for external decay. Wood, September pages 35-37.

_____ 1967b. The remedial treatment of telephone and electric transmission poles. Part 2. Treatment for internal decay. Wood October. pages 37-40.

_____ 1967c. The remedial treatment of telephone and electric transmission poles. Part 3. Treatment for internal decay. Wood November. pages 29-31.

Slaughter, J. C. 1988. Sulfur compounds in fungi. Symposium of the British Mycological Society held at the University of Birmingham. (Lynne Boddy, R. Marchant & D.J. Read. Ed.) Cambridge University Press. Cambridge, United Kingdom. pages 91-105.

Thorn, G.D., and R.A. Ludwig. 1962. The dithiocarbamates and related compounds. Elsevier Publishing Company, Ed. Amsterdam, New York. 298 pp.

Turner, N.J. 1962. Decomposition of sodium N-methyl dithiocarbamate, vapam, in Soil. PhD. Thesis. Oregon State University. Corvallis, OR. 45 p.

Turner N. J. and M. E. Corden. 1963. Decomposition of sodium N-methyldithiocarbamate in soil. Phytopathology 53:1388-1394.

Wainwright, M. 1988. Inorganic sulfur oxidation by fungi. Nitrogen, phosphorus and sulfur utilization by fungi. Symposium of the British Mycological Society held at the University of Birmingham. (Lynne Boddy, R. Marchant & D.J. Read. Ed.) Cambridge University Press. Cambridge. United Kingdom. pages 71-89.

Waksman, S. A. 1918. The importance of mould action in soil. Soil Science 6:137-135.

Wang, C. J. K. and R. A. Zabel. 1990. Identification manual for fungi from utility poles in the eastern United States. American Type Culture Collection. Allen Press Inc. Lawrence, Kansas. 356 p.

Zabel, R. A., and J. J. Morell. 1992. Wood microbiology. Decay and its prevention. Academic Press Inc. New YorK. 476 p.

Zabel, R. A.; F. F. Lombard, and A. M. Kenderes. 1980. Fungi associated with decay in treated Douglas-fir transmission poles in the northeastern United States. Forest Products Journal 30:51-56.

Zabel, R. A.; F. F. Lombard; C. J. K. Wang, and F. Terracina. 1985. Fungi associated with decay in treated southern pine utility poles in the eastern United States. Wood and Fiber Science 17(1):75-91.

Zabel R. A. and C. J. K. Wang. 1988. Utility pole problems in the eastern United States: changing viewpoints. Proceedings, The Wood Pole Conference II, Portland, OR.

Zahora, A. 1983. Methylisothiocyanate as a wood fumigant: Fungitoxicity to *Poria carbonica* in wood and gelatin encapsulation for use in wood products. M.S. thesis. Oregon State University, Corvallis, OR. 65 pp.

Zahora, A. 1987. Interactions of the fumigant methylisothiocyanate with Douglas-fir wood and their influence on fumigant effectiveness. Ph.D. Dissertation Oregon State University, Corvallis, OR.

Zahora, A.R. and M.E. Corden. 1985. Methylisothiocyanate fungitoxicity to *Poria carbonica* in Douglas-fir Heartwood. Material und Organismen 20:193-204.

Zahora, A.R. and J.J.Morrell. 1988. Decomposition of methylisothiocyanate in Douglas-fir heartwood. Forest Products Journal 38(10):46-52.

Zahora, A.R.and J.J. Morrell. 1989a. Diffusion and sorption of the fumigant methylisothiocyanate in Douglas-fir wood. Wood and Fiber Science 21(1):55-66.

Zahora, A.R. and J.J. Morrell. 1989b. The influence of wood moisture content on the fungitoxicity of methylisothiocyanate in Douglas-fir heartwood. Wood and Fiber Science 21(4):343-35.