EFFECT OF MIXTURES OF CARBON DISULFIDE AND METHYLISOTHIOCYANATE ON SURVIVAL OF WOOD-COLONIZING FUNGI

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ABSTRACT

The fungitoxicity of carbon disulfide (CS_2), methylisothiocyanate (MITC), or a mixture of these two gases, to selected wood-degrading fungi was studied by using a fumigation apparatus. Both gases are important decomposition products of metham sodium, the most commonly used fumigant for internal treatment of large wood members. Carbon disulfide (up to 8,000–9,000 ppm) was mildly toxic to most of the test fungi, and MITC (up to 18 ppm) was uniformly toxic. A combination of sublethal levels of both gases (3,000–4,000 ppm CS_2/5 ppm MITC) was more toxic than either chemical alone. The results suggest a synergism between various metham sodium decomposition products, and this interaction may account for the protection afforded by this treatment. Further studies of other decomposition products are suggested.

Keywords: Fumigant, metham sodium, carbon disulfide, methylisothiocyanate, fungitoxicity.

INTRODUCTION

Fumigants are widely used in North America to arrest and prevent internal decay of electric utility poles and other large wood members (Goodell and Graham 1983). Of these fumigants, metham sodium (32.7% sodium n-methyldithiocarbamate) is the most commonly used (Morrel and Corden 1986). This chemical itself is not highly fungitoxic, but it decomposes in the presence of organic compounds to produce a variety of highly fungitoxic compounds including methylisothiocyanate (MITC) (Turner and Corden 1963; Elson 1966). The toxicity of MITC to wood-degrading fungi has been the subject of extensive study, and its behavior under varying regimes is well documented (Zahora and Corden 1985; Zahora and Morrell 1988, 1989a, b). Metham sodium decomposition in soil produces significant levels of MITC under a variety of conditions, although various other decomposition products are also possible (Thorn and Ludwig 1962; Turner and Corden 1963; Elson 1966; Smelt and Leistra 1974).

A number of studies suggest that MITC is a relatively minor component of metham sodium decomposition in wood, with substantial amounts of carbon disulfide (CS_2) and carbonyl sulfide produced (Miller and Morrell 1990; Lebow and Morrell 1993; Morrell 1994). Although the latter two compounds can be fungitoxic, the levels required far exceed those produced by metham sodium decomposition. Futhermore, because these compounds have

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TABLE 1. *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
Gloeophyllum trabeum (Fr.) Murr.	MAD-617	FPL, Madison, WI
G. saepiarium (Fr.) Karst	S4UT	FRL, Corvallis, OR
Trametes versicolor (L.:Fr.) Pilát.	A4CD-34	FRL, Corvallis, OR
Hormoconis resinae v. Arx & de Vries	P1600	SUNY College of Env. Sci. & For., Syracuse, NY

no substantial interactions with the wood, they remain in the wood for only short periods after treatment. Despite these characteristics, metham sodium provides good protection to Douglas-fir poles over a 7- to 10-year period (Helsing et al. 1984) and to southern pine poles for 3 to 5 years (Zabel and Wang 1988). This performance may reflect synergistic interactions between various decomposition products that enhance fungitoxicity of the treatment; however, there is little information to support this premise. In this report, we describe the effects of CS_2 and MITC, alone or in combination, on survival of seven wood-colonizing fungi.

MATERIALS AND METHODS

Blocks were colonized by six basidiomycetes and one ascomycete by using modifications of a previously described procedure (Sexton et al. 1993/94) (Table 1). Blocks (10 by 10 by 3 mm) of Douglas-fir heartwood (*Pseudotsuga menziesii* (Mirb.) Franco) and ponderosa pine sapwood (*Pinus ponderosa* Dougl. ex Laws.) 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, and the bags were loosely sealed prior to autoclaving for 20 min at 120 C. Each bag was then inoculated with a macerated hyphal/spore mixture of one of the seven fungi.

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, and then the mycelium was filtered, resuspended in sterile distilled water, and blended for 10 sec 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 a plastic bag, and the bags were heat sealed and incubated 20 to 30 days at 27 C. The ascomycete, *H. 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 suspensions. 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 colonized blocks were exposed to metham sodium decomposition products in a fumigation apparatus consisting 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 connected to a manifold that distributed the gas mixture to 12 135-ml glass jars. Each jar contained 10 blocks colonized by a single fungus. Flow from the fumigant jars to the mixing chamber was

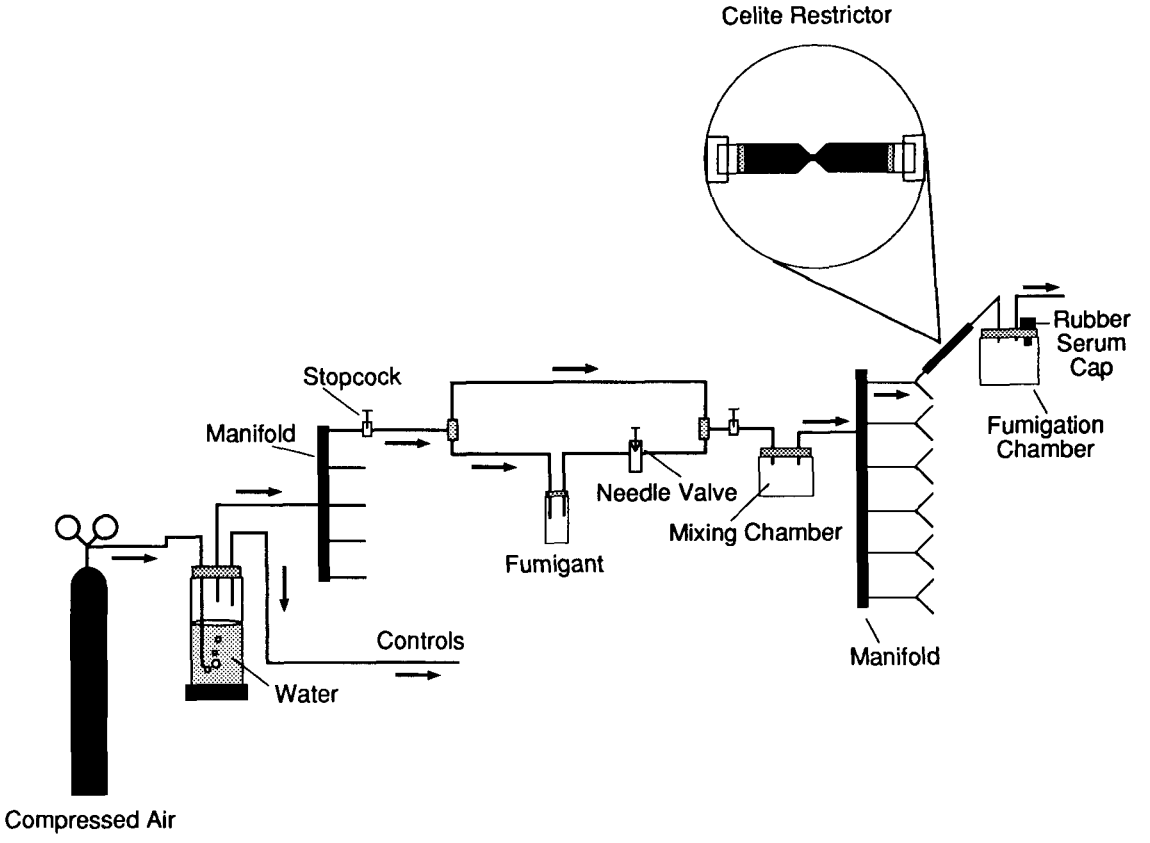

FIG. 1. Apparatus employed to fumigate fungus-colonized wood blocks.

controlled with Teflon-lined control valves, while flow to individual fumigation chambers was controlled through glass restrictor tubes packed with Celite® (diatomaceous earth) to produce a flow of 15 ml of fumigant-laden air per minute. All gas flows were measured with 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 being bubbled through distilled water. Then the air flowed over the jars containing the fumigant, through the mixing jar, and finally into the jars that would contain the blocks. Fumigant flow rates were adjusted until the desired concentration of each gas was achieved, and then the apparatus was allowed to operate for 24 h 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 analysis of CS_2, 5 μl of air was injected into a Varian 3700 Gas Chromatograph (GC) equipped with a Flame photometric detector with filters specific for sulfur. The GC conditions were as follows: nitrogen flow rate 33.3 ml/minute; detector temperature 240 C; injector temperature 150 C. Column temperature was 40 C. Separation was achieved with a 3-m by 4-mm (inner diameter) column packed with 10% Carbowax 20M on 80/100 Supelcoport (Supelco, Bellefonte, PA). For MITC, 200-μl air samples were injected; GC

conditions were similar except that column temperature was increased to 110 C.

The fumigant chamber was first employed to evaluate the fungitoxicity of CS_2 at 500, 3,000–4,000, and 8,000–9,000 ppm (0.5, 3–4, and 8–9 ng of $CS_2/\mu l$ of air, respectively) or MITC at 5, 10, and 18 ppm (5, 10, and 18 ng MITC/ml of air). The results from these trials indicated that 3,000–4,000 ppm CS_2 and 5 ppm MITC were sublethal exposure rates, and the effects of a mixture of these two gases at the sublethal level were then evaluated.

Fumigations were carried out over 10-day periods. Each day, one colonized block 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 a stainless steel ball and 5 ml of sterile distilled water. The canister was shaken for 5 seconds on a Kleco® 4100 Pulverizer (Kleco Kinetic Manufacturing Co., Visalia, CA). After maceration, the steel ball was removed with 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 C) 1.8% malt extract agar, and the mixture was poured into petri dishes and allowed to solidify. Three plates were prepared from each block. The plates were incubated at room temperature, and the colonies that developed in each plate were counted. These results were compared with colony counts from control blocks exposed to air flow without fumigant through a separate line in the fumigation apparatus (Fig. 1). The data were expressed as number of colony-forming units (CFUs) in order to reflect the possibility that colonies could arise from both hyphal fragments and spores. The data were used to construct concentration × time (CT) curves for assessing the amount of chemical necessary to kill each fungus at selected exposure times. The CT values necessary to kill 90% of propagules (CT_{90}) were calculated from the regression lines on these curves.

RESULTS AND DISCUSSION

The number of CFUs varied widely among the fungal species, reflecting both the sensitivity of each species to the isolation procedures and the presence or absence and relative amounts of conidia or chlamydospores in the wood (Tables 2–4). *Irpex lacteus,* which produced the smallest number of CFUs, 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). *Hormoconis resinae* produced the largest number of CFUs, reflecting the massive sporulation that is a common feature of this species (Bessey 1959).

CFUs 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 in the test apparatus through distilled water before it passed over the blocks. Condensation along the walls of the tubing indicated that moist air was reaching the blocks, but signs of dryness appeared after 7 to 8 days. The CFU declines in controls may reflect loss of viability due to drying during the exposure period, although declines did not always occur. For example, CFUs of *A. carbonica* growing on pine increased with time. Despite declines in some species, differences between chemically exposed samples and controls were generally of a magnitude that permitted comparisons between the treatments.

Carbon disulfide

Fumigation with CS_2 appeared to stimulate the number of CFUs in most of the treatments between the first and fourth days of exposure (Table 2). For *P. placenta* on Douglas-fir, increases in CFUs up to 263% (for 500 ppm) and 420% (8,000–9,000 ppm) were obtained. For the same species on pine, increases ranged from 182% (500 ppm) to 370% (8,000–9,000 ppm). Many fungi produce thick-walled chlamydo-

spores that are able to withstand long exposures to toxic substances or to other adverse environmental conditions (Zabel and Morrell 1992). *Antrodia carbonica* produced large amounts of thick-walled chlamydospores, but *I. lacteus,* which does not produce this type of structure, was less tolerant of the treatments.

The lowest level of CS_2 (500 ppm) had little effect on CFUs for any of the species tested. CFUs for *P. placenta, G. saepiarium, T. versicolor,* and *H. resinae* exceeded 100% after 10 days of fumigation. Some fungi growing under sulfur-deficient regimes have been shown to be more resistant to sulfur-containing fumigants and even stimulated when fumigated for 2 h with MITC (Cobb 1972). 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 content in wood is generally less than 0.1% for species such as Douglas-fir and pine (Mingle and Boubel 1968). It is possible that some fungi metabolize CS_2 or MITC to satisfy sulfur needs. These effects may help explain the initial increase in CFUs observed for most of the species fumigated with CS_2, although more refined studies of sulfur balance in fumigated fungi would be required to confirm this hypothesis.

Increasing CS_2 levels to 3,000–4,000 ppm produced declines in CFUs for virtually all of the fungi tested, but this level was only effective against *I. lacteus,* which produced no viable CFUs at the end of the treatment.

Exposure to 8,000–9,000 ppm of CS_2 resulted in decreased CFUs for all of the fungi tested, although many species survived a 10-day exposure. Initial stimulation of CFUs was again noted for *A. carbonica, P. placenta,* and *G. saepiarium* on both Douglas-fir and pine, and for *I. lacteus* and *T. versicolor* on pine. Prolonged exposure resulted in very low levels of survival for *A. carbonica* on pine, *P. placenta, I. lacteus,* and *G. trabeum* on both Douglas-fir and pine, *G. saepiarium* on Douglas-fir, and *T. versicolor* on Douglas-fir, suggesting that the fumigant might be effective upon even longer exposures. Drying of wood in the fumigation system at these longer exposures, however, would cause a corresponding decrease in CFUs, obscuring potential fumigation effects. Several fungi, including *A. carbonica* on Douglas-fir, *G. saepiarium* on ponderosa pine, *T. versicolor* on pine, and *H. resinae* on Douglas-fir and ponderosa pine, were relatively unaffected by this exposure. *Antrodia carbonica* is an important colonizer of Douglas-fir heartwood, and the survival of this species in the presence of CS_2 would be a major drawback if this fumigant were the only decomposition product of metham sodium (Eslyn 1970; Graham and Corden 1980). Similarly, *G. saepiarium* is an important colonizer of untreated pine, although studies suggest that it is not an important colonizer of preservative-treated southern pine (Zabel et al. 1980). The survival of *H. resinae* at the highest CS_2 level is not surprising in light of the well-known tolerance of this species to a variety of biocides. This fungus occasionally has been isolated from Douglas-fir poles (Zabel et al. 1980), but in creosote-treated southern pine utility poles, it was the most frequently isolated fungus (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 CS_2 is unknown (Marsden 1954; Kerner-Gang 1976).

These results demonstrate that exposure of wood colonized by various decay and nondecay fungi to low levels of CS_2 reduces the number of CFUs in the wood, but fumigants at these levels generally were not lethal. Metham sodium is a relatively short-lived treatment, with reinvasion of poles by decay fungi characteristically starting only 3 to 7 years after treatment (Helsing et al. 1984; Zabel et al. 1985; Zabel and Wang 1988). Metham sodium's limited residual time 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,

TABLE 2. *Effect of fumigation with CS_2 on number of colony-forming units (CFUs) produced by seven species of fungi on Douglas-fir or ponderosa pine, expressed as a % of controls.*[a]

Time (days)	*A. carbonica*		*P. placenta*		*I. lacteus*		*G. trabeum*		*G. saepiarium*		*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 CS_2							
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	106	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
							3,000–4,000 ppm CS_2							
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	15	57	4	3	0	134	19	68	68	76	84	83	67
9	55	14	49	3	1	0	126	14	69	69	89	69	73	69
10	47	15	55	2	0	0	103	14	71	71	79	27	69	66
							8,000–9,000 ppm CS_2							
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	122	150	420	370	48	109	90	32	126	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.

or other wood defects. If decomposition conditions shift heavily towards production of CS_2, some fungi may survive as chlamydospores and later be able to germinate when conditions again become suitable for microbial growth.

Fungitoxicity of MITC

As expected, MITC had a more dramatic effect than CS_2 on CFU numbers for all fungal species in the study (Table 3). Like CS_2, MITC often produced an initial stimulus of CFUs during the first 2 days of fumigation, especially in the 5 and 10 ppm MITC treatments.

The lowest concentration (5 ppm) produced declines in CFUs for all species except *T. versicolor,* which showed significant increases in CFUs after the fifth or sixth day on both Douglas-fir and pine (Table 3). This effect may reflect delayed stimulation under low fumigant concentrations, but reasons for the lag are un-

TABLE 3. *Effect of fumigation with MITC on number of colony-forming units (CFUs) produced by seven species of fungi on Douglas-fir or ponderosa pine, expressed as % of controls.*[a]

Time (days)	*A. carbonica* D-fir[b]	*P. placenta*		*I. lacteus*		*G. trabeum*		*G. saepiarium*		*T. versicolor*		*H. resinae*	
		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	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	10	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.
[b] Data for *A. carbonica* on ponderosa pine was lost because of contamination.

clear. Relatively high CFU levels were found for *A. carbonica* on Douglas-fir and *P. placenta* on pine. (The data for *A. carbonica* on pine were lost during MITC fumigations because of contamination.) Little or no fungal survival on either wood was found for *I. lacteus, G. trabeum, G. saepiarium,* and *H. resinae.* Differences in the number of CFUs among species (Table 3) reflect the different fungi's abilities to react to environmental changes, especially with regard to numbers and types of resistant structures. For example, *G. trabeum* and *G. saepiarium* were characterized by high CFU counts at the beginning of fumigation, but these levels declined rapidly with time, suggesting that these species were very susceptible to fumigation under the conditions of this experiment. Both species produce large numbers of

TABLE 4. *Effect of fumigation with 3,000–4,000 ppm CS_2 and 5 ppm MITC on number of colony-forming units (CFUs) produced by seven species of fungi on Douglas-fir or ponderosa pine, expressed as % of controls.*[a]

Time (days)	*A. carbonica* D-fir[b]	*P. placenta*		*I. lacteus*		*G. trabeum*		*G. saepiarium*		*T. versicolor*		*H. resinae*	
		D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine
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.
[b] Data for *A. carbonica* on ponderosa pine was lost because of contamination.

thin-walled arthrospores, which may be less resistant to MITC fumigation than chlamydospores. However, the presence of even limited surviving CFUs could facilitate fungal "reinvasion" far from the fumigant application point, where MITC levels might be expected to be low. The possibility of a surviving microflora in fumigated 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 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 of CFUs for all species (Table 3). On both Douglas-fir and pine, *I. lacteus, G. trabeum,* and *G. saepiarium* showed rapid declines in CFUs until the seventh to ninth days, when these species succumbed. As in the CS_2 and 5 ppm MITC fumigations, initial increases in CFU levels were evident in most species during the first days of treatment, but the levels were much lower. Only *A. carbonica* on pine, *P. placenta* and *T. versicolor* on Douglas-fir, and *H. resinae* on both woods survived a 10-day exposure to 10 ppm MITC, and even these species experienced marked CFU declines compared with results in the CS_2 and 5 ppm MITC treatments.

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

Fungitoxicity of the CS_2/MITC mixture

Because both CS_2 and MITC are produced during metham sodium decomposition, these products may act synergistically to enhance fungal control. The initial results suggested that 3,000–4,000 ppm CS_2 and 5 ppm MITC each produced CFU declines that were usually noticeable but not lethal. These levels were subjected to further study in combination.

The initial CFU stimulus noted with CS_2 or MITC alone was absent in the mixture; as before, however, sampling was performed every 24 h, and a stimulus earlier in the fumigation might have been missed by our procedures. Mixtures of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) were generally more fungitoxic than CS_2 alone at 8,000–9,000 ppm or MITC alone at 10 ppm for *A. carbonica, P. placenta, I. lacteus,* and *G. trabeum* (Table 4). *T. versicolor* had shown high CFUs for the 5 ppm MITC fumigation, but this effect was less pronounced with the mixture. Fumigant mixtures did not noticeably reduce the CFUs of *G. saepiarium* or *H. resinae* compared

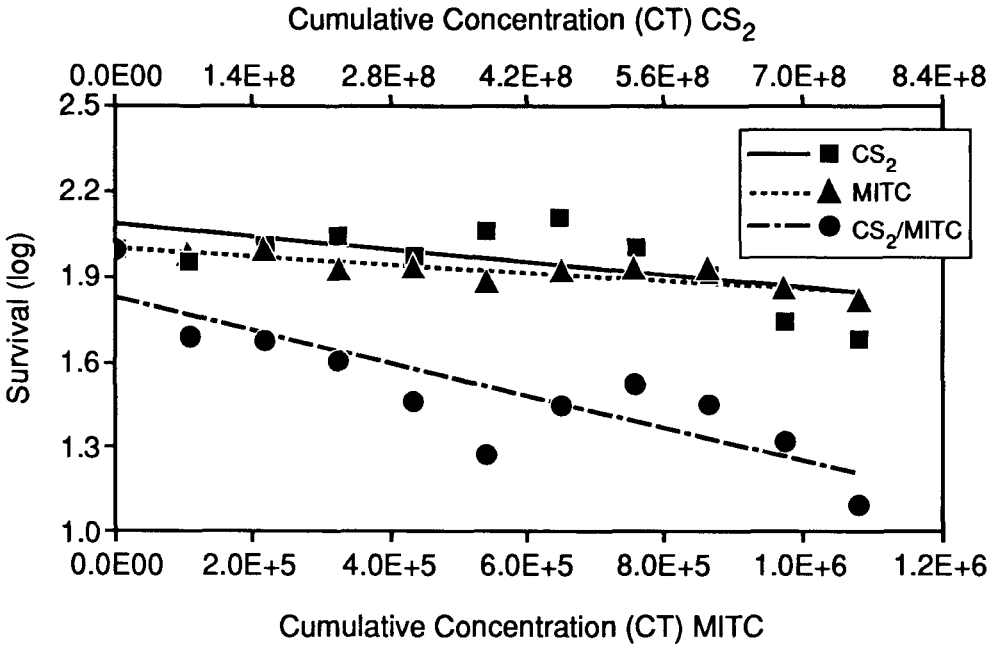

FIG. 2. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *A. carbonica* on Douglas-fir. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

with the 5 ppm MITC treatment, but the mixture was more effective than CS_2 alone at 3,000–4,000 ppm.

Concentration × time (CT) estimates

Although air concentration of a chemical is a useful measure of fumigant exposure, fumigant studies are more often expressed as concentration × time or CT values. These values reflect the total dosage to which the fungus was exposed. The CT required to reduce CFUs to 10% of the control, or CT_{90}, provides a useful measure of chemical effectiveness.

CT curves for sublethal dosages of each chemical alone or in mixture show that for most fungi, the log of survival declined more rapidly with the mixture than with either fumigant alone (Figs. 2–8). The effects were more variable for *T. versicolor* (Fig. 7), where the relationship between CT and survival changed at lower CT values. These effects again reflect the initial stimulation of CFUs previously discussed.

For most fungal species, CT_{90} values for the fumigant mixture were generally lower than those for either CS_2 or MITC at sublethal levels (Table 5). For example, CT_{90} values for the mixture against *A. carbonica* on Douglas-fir were less than one third the value for CS_2 alone, or about a fifth the value for MITC (5 ppm) alone. The CT_{90} for the mixture for *P. placenta* on Douglas-fir was about a third of the value for CS_2 or MITC alone; on pine, effects of the mixture were similar to those of CS_2. In some instances, however, CT_{90} values for the mixture were higher than those for the individual components. For *I. lacteus* on pine, CT_{90} values for the mixture were half again as large as for CS_2 alone. For *G. saepiarium* on Douglas-fir, CT_{90} values for the mixture were higher than for MITC. For *H. resinae* on Douglas-fir or pine, the CT_{90} for the mixture was similar to that found for sublethal levels of MITC (5 ppm).

CT_{90} values for the 3,000–4,000 ppm CS_2 treatment tended to be higher for Douglas-fir

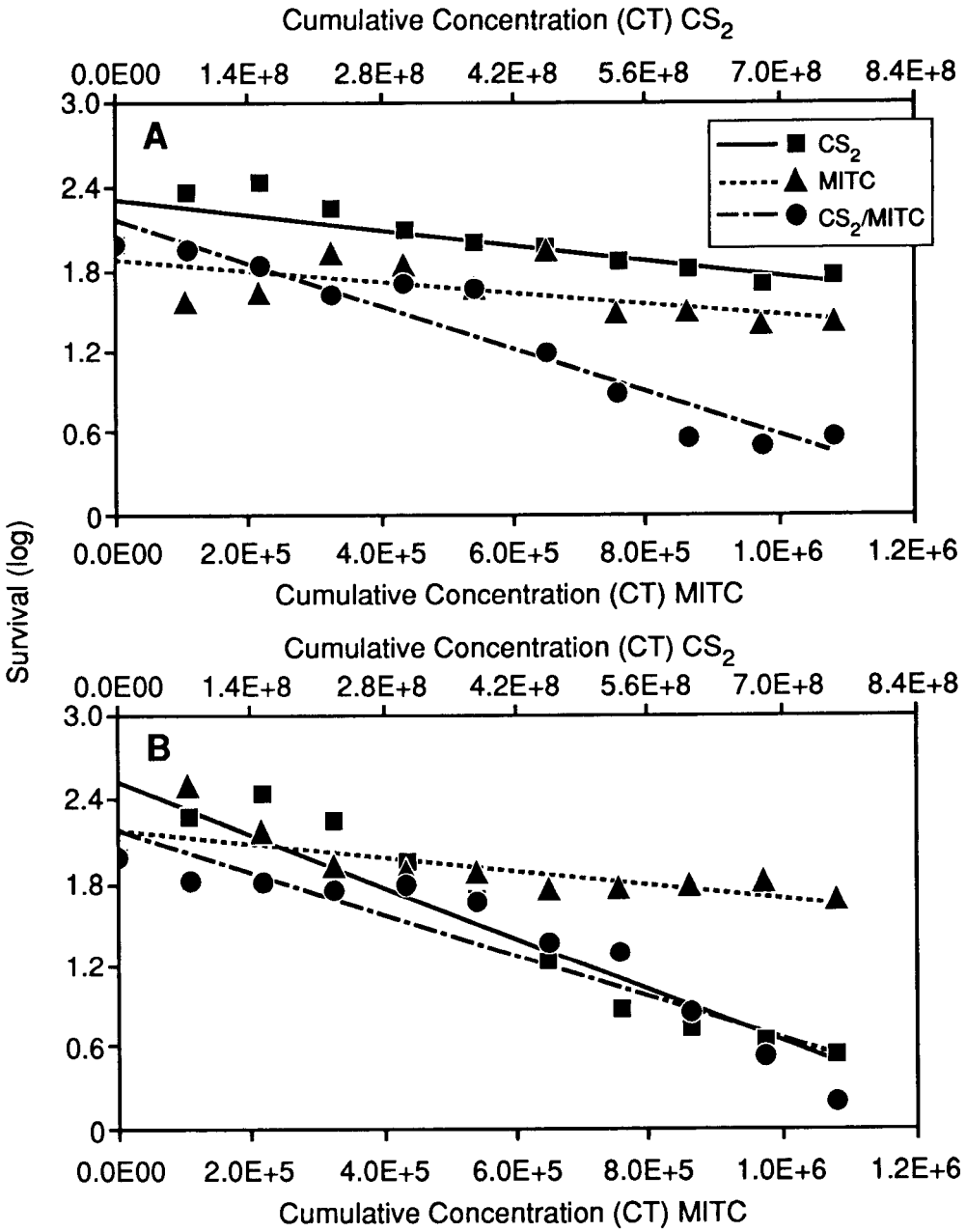

FIG. 3. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *P. placenta* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

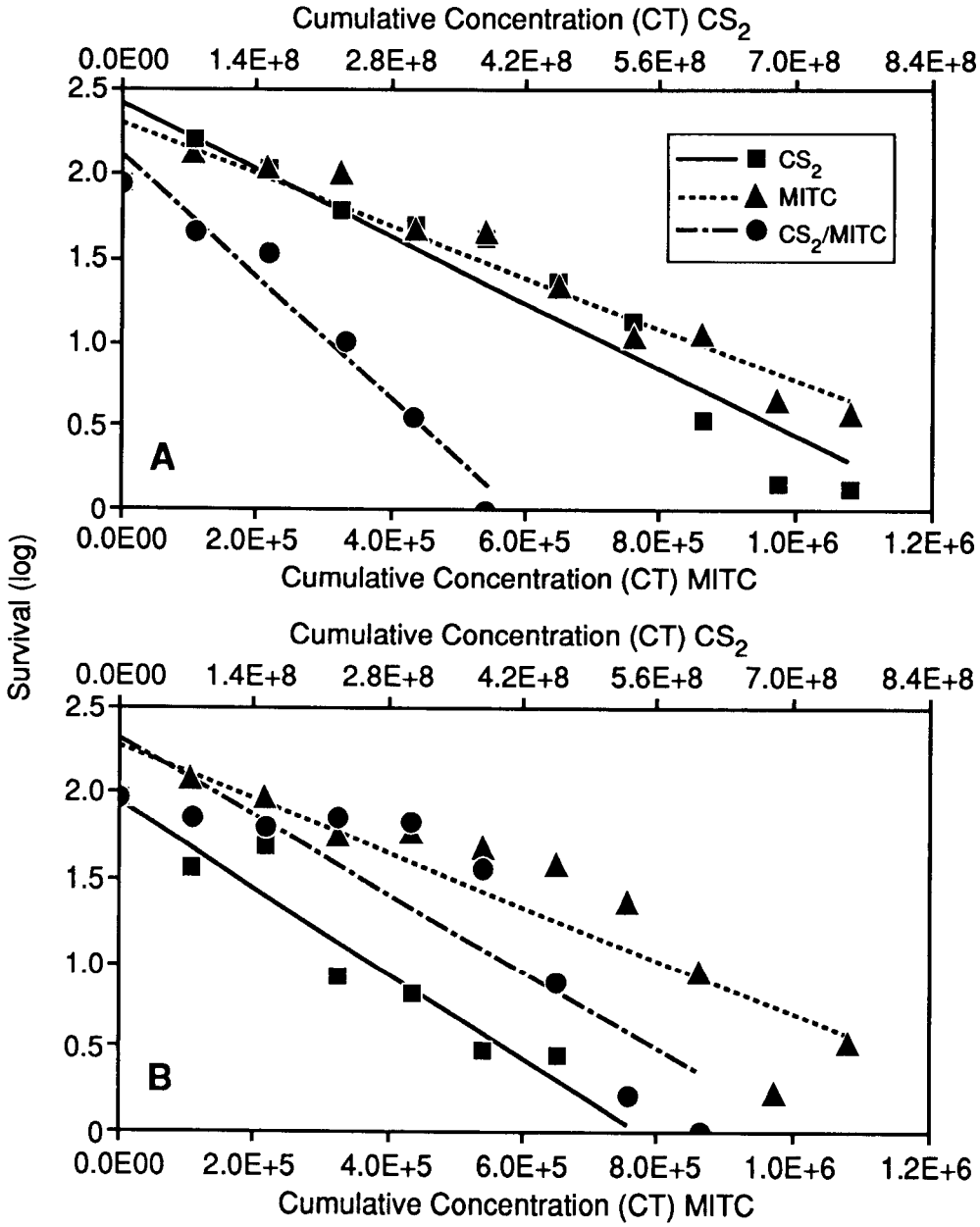

FIG. 4. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *I. lacteus* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

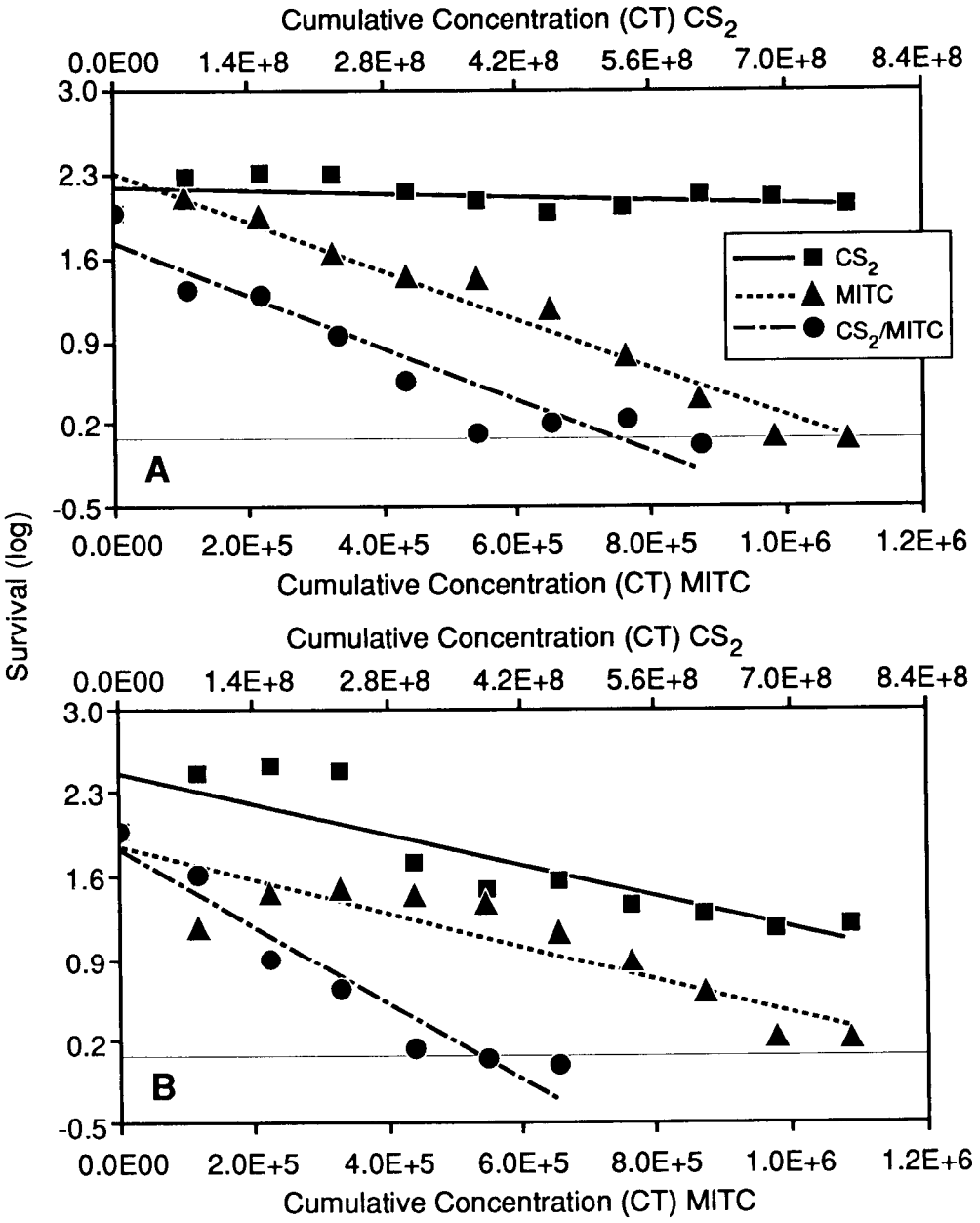

FIG. 5. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *G. trabeum* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

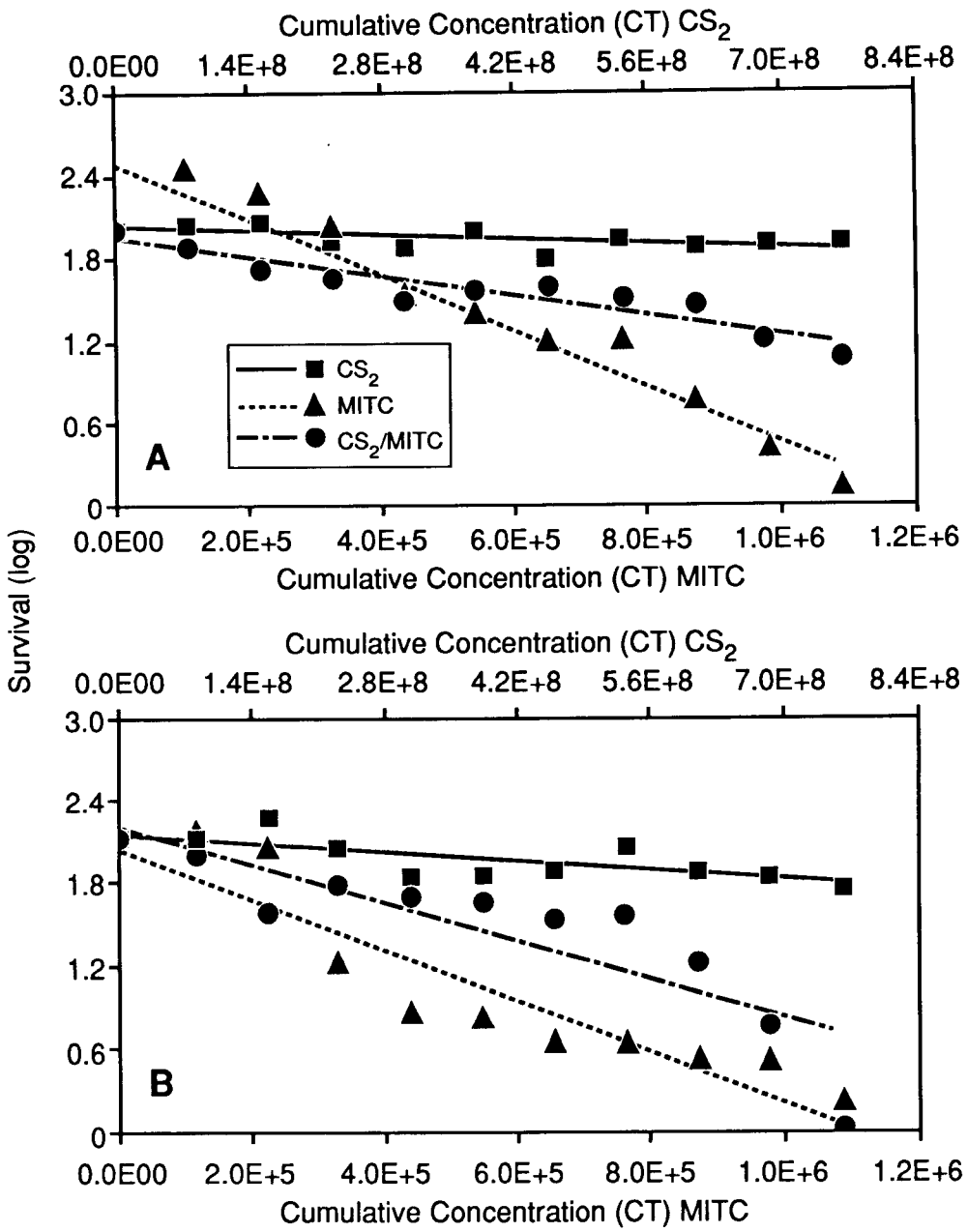

FIG. 6. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *G. saepiarium* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

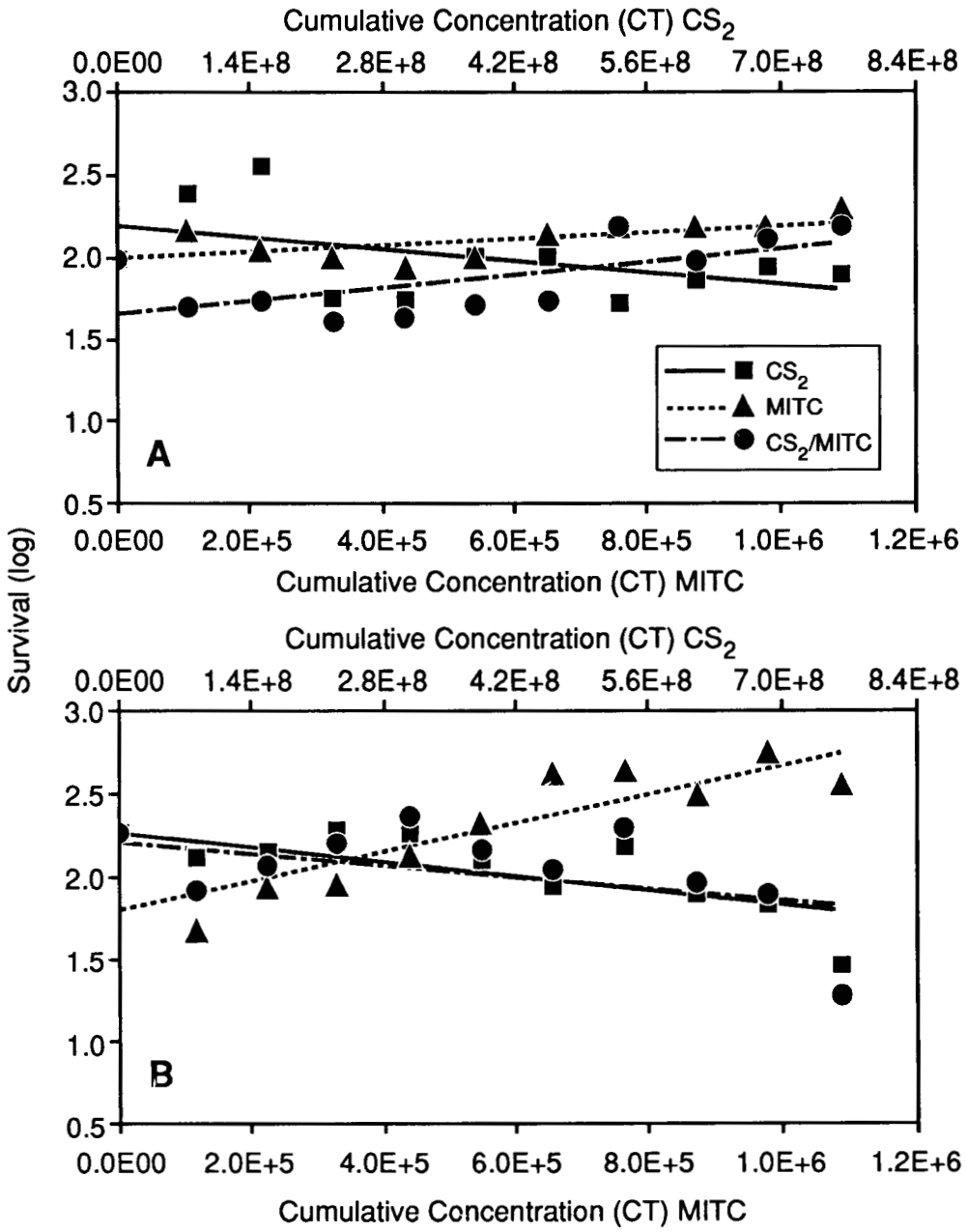

FIG. 7. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *T. versicolor* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

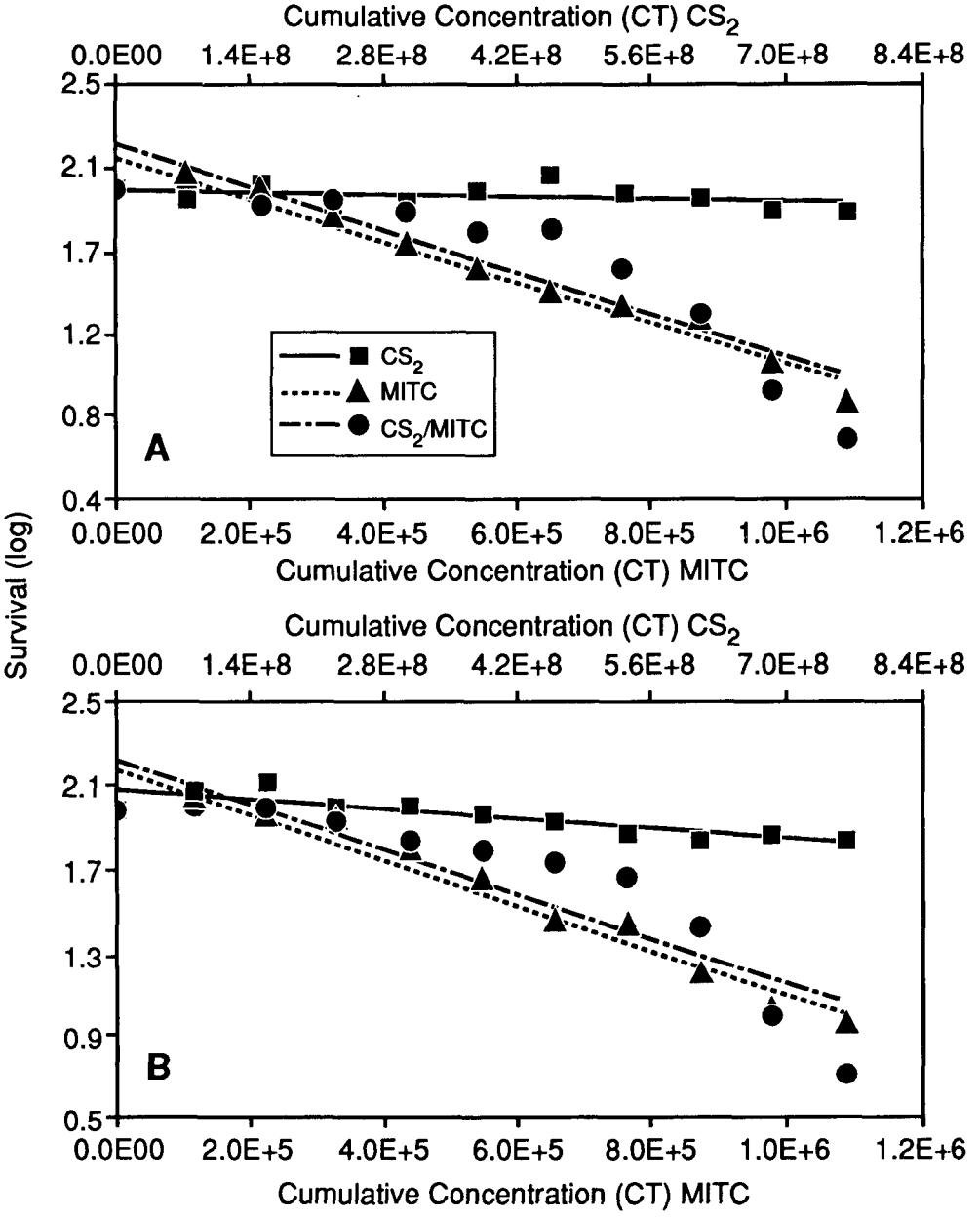

FIG. 8. Effect of sublethal dosages of CS_2 (3,000–4,000 ppm) and MITC (5 ppm) and a CS_2/MITC mixture (3,000–4,000 ppm CS_2/5 ppm MITC) on survival of *H. resinae* on A) Douglas-fir and B) ponderosa pine. The Y axis represents the logarithm of survival, and the X axes represent the product of concentration by time for CS_2 (upper axis) and MITC (lower axis).

TABLE 5. *Concentration × time values necessary to kill 90% of the propagules (CT_{90}) for seven species of fungi exposed to CS_2, MITC, or a CS_2/MITC mixture. (CT_{90} values are all $\times 10^6$.) Values in parentheses represent the number of days necessary to reach 90% kill; for the mixture, values in parentheses are for the combined CS_2 and MITC treatment.*

	A. carbonica D-fir[b]	*P. placenta*		*I. lacteus*	
		D-fir	Pine	D-fir	Pine
CS_2					
3,000–4,000 ppm	3,172 (42)	1,544 (20)	551 (7)	489 (6.5)	253 (3)
8,000–9,000 ppm	3,497 (19)	1,722 (9)	1,193 (6.5)	780 (4)	798 (4)
MITC					
5 ppm	6.72 (62)	1.95 (18)	2.21 (20.5)	0.83 (7.7)	7.85 (72)
10 ppm	7.47 (35)	1.99 (9)	1.81 (8.4)	1.10 (5)	1.05 (5)
18 ppm	1.99 (5)	1.3 (3.3)	1.67 (4.3)	1.16 (3)	1.12 (3)
Mixture					
3,000–4,000 ppm CS_2	942	496	526	208	391
5 ppm MITC	1.35 (2.5)	0.71 (6.6)	0.75 (7)	0.33 (3)	0.56 (5)

than for ponderosa pine, but this effect was not evident for higher CS_2 levels (8,000–9,000 ppm), for MITC alone, or for the CS_2/MITC mixtures. Douglas-fir heartwood is less permeable than pine sapwood (Panshin and DeZeeuw 1980); however, Douglas-fir permeability to MITC is largely influenced by wood moisture content (Zahora 1987). Very low MITC concentrations, which may not be toxic to inactive decay fungi in dry wood, become fungitoxic in wet wood. Because fungal growth and active decay will only occur in wood above the fiber saturation point, increased susceptibility of *A. carbonica* to MITC in wet wood may be important in determining long-term wood protection. In the present study, moisture content in wood was maintained above the fiber saturation point, which may help explain the excellent fungitoxicity of the fumigants despite the low MITC concentrations employed.

CONCLUSIONS

The results from the present study suggest a synergism between CS_2 and MITC that enhances metham sodium fungitoxicity. This synergism, if present between other metham sodium decomposition products, may help explain the relatively strong performance of this fumigant in wood.

The interactive effects of CS_2 and MITC suggest that the fungicidal action of metham sodium in wood is far more complex than previously thought. While synergistic activity among biocides is well documented, the possibility that the various metham sodium decomposition products can interact to enhance performance helps explain the higher activity of this fumigant compared with that of others. The results also suggest that interactions may occur among the other decomposition products. Metham sodium decomposes to more than 14 possible compounds. Of these, only CS_2 and MITC 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 that maximize the fungitoxicity of metham sodium in wood; such factors could be used to enhance decomposition to produce the compounds most likely to affect fungal control.

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TABLE 5. *Extended.*

G. trabeum		*G. saepiarium*		*T. versicolor*		*H. resinae*	
D-fir	Pine	D-fir	Pine	D-fir	Pine	D-fir	Pine
5,337 (70)	764 (10)	3,726 (49)	2,258 (30)	2,313 (30)	2,010 (27)	7,485 (100)	2,819 (37)
1,770 (9.7)	1,374 (7.5)	1,237 (7)	2,916 (16)	1,670 (9)	3,141 (17)	4,872 (26)	2,539 (14)
0.61 (5.6)	0.57 (5)	0.69 (6)	0.54 (5)	NA	NA	1.03 (9.5)	1.03 (9.5)
1.01 (5)	1.06 (5)	1.03 (5)	0.83 (4)	2.60 (12)	1.44 (6.7)	1.60 (7.4)	1.49 (7)
1.17 (3)	1.31 (3.4)	0.96 (2.5)	0.82 (2)	1.93 (5)	1.93 (5)	1.98 (5)	1.83 (4.7)
211	167	842	571	NA	2,299	739	760
0.30 (3)	0.07 (2)	4.20 (11)	0.82 (7.5)	NA	3.28 (30)	1.06 (10)	1.09 (10)

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